Vol. XVII OCTOBER, 1931 No. 1

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Pathology
PAUL G. WOOLLEY, M.D.
Pasadena

Physiology
J. J. R. MACLEOD, M.B.
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
University of Pennsylvania, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS.

Entered at the Post Office at St. Louis, Mo., as Second Class Matter.

DR. WALTER W. MURPHY, Minneapolis, Minnesota, became a member of the P. C. A. March 26, 1930, and on June 1, 1931, was instantly killed when his car skidded on a slippery curve, going over a bank and landing on a railroad track forty feet below. The $5,000.00 paid his sister had cost Dr. Murphy $17.00.

Since January 1, 1931

$339,256.18

has been paid to Physicians and Dentists for sick and accident claims, of which $45,000.00 was for fatal accidents.

Thirtieth year of successful operation under same management as purely mutual, non-profit, societies. $200,000.00 State deposit for the protection of all members, wherever located.

COST [$13.00 yearly for each $5,000-$25 accident policy.
$17.00 yearly for each $25.00 weekly health policy.]
Payable quarterly if desired.

One, two, or three policies in each Association may be issued to one person

PHYSICIANS CASUALTY ASSOCIATION—PHYSICIANS HEALTH ASSOCIATION
OMAHA E. E. ELLIOTT, Sec.-Treas. NEBRASKA

BRING YOUR GLASS PROBLEMS TO

HARRIS COMER

STEELE GLASS COMPANY

145-147 NORTH FOURTH STREET

GLASS APPARATUS GLASS BLOWING

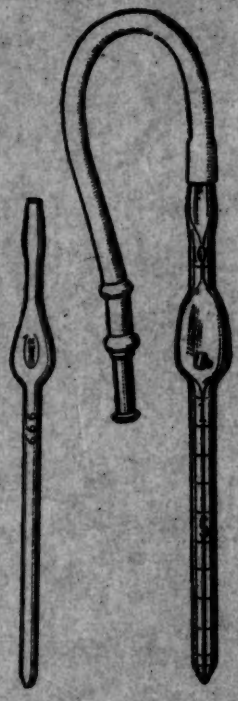
FOR CHEMICAL, RESEARCH, BACTERIOLOGICAL LABORATORIES

We offer the service of skilled workmen and our experience of 40 years in adapting glass to a multitude of scientific and industrial purposes

PHONE LOMBARD 5856

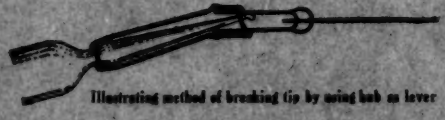
GLASS TUBE GLASS ROD

TRADE COMER MARK



comer

This trade-mark on the back of our Blood Count Pipette means that we guarantee a Greater Accuracy than is required by the U. S. Bureau of Standards.



Illustrating method of breaking tip by using hub as lever

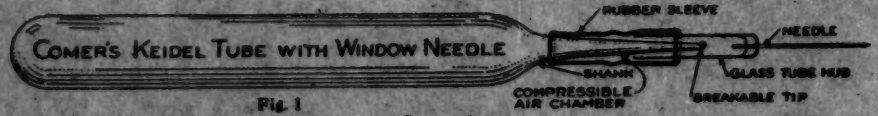


Fig. 1

Patented

Comer's Window Needle *makes it possible to SEE that*

- The breakable tip of ampoule is not prematurely broken.
- The Blood shows in Glass Hub when needle is properly introduced into vein.

BLOOD CHEMISTRY KEIDELS

with Sodium Fluoride as used by the Pennsylvania Department of Health under the direction of Dr. John L. Laird

---

HARRIS COMER — PHILADELPHIA, PA.

Medical
Main

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., OCTOBER, 1931 No. 1

CLINICAL AND EXPERIMENTAL

PHYSIOLOGY, CORRELATIONS, AND TECHNIC OF THE VAN DEN BERGH REACTION, ICTERUS INDEX, AND QUANTITATIVE SERUM BILIRUBIN*

BY NORMAN W. ELTON,† M.D., D.N.B., BOSTON, MASS.

MANN and Rich have shown that if the liver of a dog is extirpated, intense tissue jaundice rapidly develops. Mann found that the beginning of this icterus may be detected in the serum of the animal as early as fifteen minutes following hepatectomy. Thus the extrahepatic origin of bilirubin in a mammal is demonstrated, as well as the rate at which it is produced. Mann also observed that the serum bilirubin obstructed by the removal of the excretory organ was direct negative in terms of the van den Bergh reaction. He further found that if, prior to hepatectomy, the common duct had been ligated long enough to cause a jaundice in which the serum contained direct positive bilirubin, the icterus again rapidly intensified after hepatectomy, but this was again due to the accumulation of direct negative bilirubin, since the level of the direct positive pigment remained unchanged following the removal of the liver. The causative factor of the jaundice in each of these experiments is obviously obstruction, the difference in the nature of the serum bilirubin being dependent on whether the site of the obstruction be placed above or below the liver parenchyma, where it has long been recognized that direct negative bilirubin is changed to the direct positive form in the mammalian organism.

The excretion of bilirubin by the liver is in many ways analogous to the excretion of the nonprotein metabolites by the kidneys. In either case the excreted substances are produced in another part of the body. When uremia exists, a disturbance in the excretory organ is postulated, and not an over-

*From the Department of Pathology, Detroit College of Medicine and Surgery, and the Fifth Medical (Boston University Teaching) Service, Boston City Hospital.
Received for publication, March 24, 1931.
†Assistant in Medicine, Boston University School of Medicine.

production of the metabolites. Bilateral nephrectomy would not be acceptable proof that the kidneys could have nothing to do with uremia. The balance between production and elimination is regulated chiefly by the efficiency of the excretory organ.

Van den Bergh states, "When more bilirubin is brought to the liver cells, more is excreted, but also more is retained by the blood . . . . if the liver cells are compelled to reject some of the excess, more may also appear in the bile . . . . this is characteristic of acholuric icterus . . . . it would not be correct to call this type of icterus nonhepatic . . . . the term 'hematogenous' icterus is incorrect, since every icterus is, in a sense, hematogenous, although bilirubin is never formed in the blood stream . . . . this form of icterus is due to insufficient capacity of the liver cells to excrete bilirubin, be it relative, following greater influx, or be it absolute due to functional liver disturbance . . . . its best name is dynamic or *functional* icterus." Thus a form of icterus commonly known as "hemolytic" is seen in the light of functional obstruction.

Von Bergmann estimates that in the average normal 70 kilo human adult the daily bilirubin production is about 500 mg., which, in terms of 100 c.c. units of blood serum volume, may be computed as approximately 14.3 mg. The normal bilirubin level in human serum does not exceed 0.2 mg. per 100 c.c., except for occasional elevations to about 0.35 mg. Visible jaundice may appear in the white race when this level rises to 1.5 or 2.0 mg. and may often be detectable in the sclerae at much lower levels. The excretion rate must be efficiently maintained to preserve the balance and prevent a constantly ascending icterus, whether the excretion threshold be normal or pathologically elevated. The elimination of bilirubin is further complicated by the evidence that during excretion it is changed from the direct negative form to the direct positive form, thus introducing a conversion mechanism as an integral part of the excretion process.

The difference between these two forms of bilirubin has been investigated by several workers, among them Blankenhorn, M'Gowan, and Collinson and Fowweather. Blankenhorn found that there was present in bile and in many ieteric sera a form of bilirubin which would pass through an animal membrane, showing that since it acted as a diffusate on dialysis, it must be in the crystalloid state. He found, however, that this was a relatively unstable form, for on repeated dialysis of the diffusates, less and less would pass through the membrane, indicating that it readily reverted to a nondialyzable, or colloidal, form. Collinson and Fowweather applied this observation to the van den Bergh reaction, and found that the crystalloid form alone gave the direct positive reaction, while the nondialyzable, or colloidal, form was direct negative, reacting with the diazonium salt only after the addition of alcohol and giving the "indirect" or quantitative reaction. M'Gowan found that the use of buffer solutions and a change in hydrogen-ion concentration toward greater alkalinity could cause a direct negative reaction to become direct positive. It is rational to conclude, then, that the difference between the

two forms of bilirubin is largely a matter of physical state: the direct negative being free bilirubin, a suspensoid colloid; the direct positive being a true crystalloid.

Sufficient data are avialable to imply that nascent bilirubin, as it is produced extrahepatically in a mammal, is in the colloidal state, and that it is converted by the liver parenchyma during excretion to an ionizable form which is a true but unstable crystalloid. This latter form has been described by different workers as an ammonium salt of bilirubin and as sodium hydrogen bilirubinate. That the liver parenchyma is ordinarily impermeable to colloids was found during the development of the Graham test, providing further evidence of the necessity for a physiologic conversion mechanism as an integral part of the excretion process for bilirubin.

The experiments of Naunyn and Minkowski, expanded by McNee, have shown that in birds the Küpffer cells play the most important rôle in the formation of bilirubin from hemoglobin, while, in contrast with mammalian physiology, the bone marrow and spleen are relatively unimportant in this respect. Since bird serum normally contains very little bilirubin, the pigment must be passed on by the Küpffer cells across the subendothelial lymphatics of the liver lobules to the polygonal cells as rapidly as it is formed. Hence, in birds a dual rôle of these cells in formation and excretion is implied. In mammals, however, it is clear from the work of Mann that the rôle of the Küpffer cells in formation of bilirubin is quite insignificant, and their only possible remaining function, if any, would be largely excretory, as the acceptors of nascent colloidal bilirubin to convey the pigment across the lymphatic spaces from the sinusoidal stream to the polygonal cells for conversion and excretion. As actively phagocytic sessile cells they are known to have an affinity for colloidal particles, although bilirubin appears to be the only colloidal substance for which the subendothelial lymphatic spaces are bridged. If such a function be granted, then their relative permeability to nascent bilirubin would determine the excretion threshold.

The direct delayed van den Bergh reaction can rationally be interpreted as indicative of the presence of bilirubin undergoing the transition from the colloidal to the crystalloid state, manifesting by the length of the delay period its proximity to one or the other of the two extremes. Colloidal particles, breaking down into polymerized, then simple, crystalloid molecules would be expected to react in an aqueous medium with a speed characteristic of their relative solubility. Many similar chemical reactions exhibit definite delay periods, as, for example, the colloidal gold reaction with spinal fluid. The source of direct delayed bilirubin would again be liver parenchyma, since it is there that the conversion takes place.

Should an obstructive process exist in or below the liver parenchyma, complete enough to produce intense pigmentary congestion in the excreting cells, much converted pigment would escape into the blood stream by way of the subendothelial lymphatic spaces of the liver lobules, but also free bilirubin, normally conveyed to the sinusoids at a rapid rate would be denied entry to the polygonal cells, resulting in an icteric serum containing both forms of bilirubin. Such sera would obviously give direct positive reactions, the direct negative

content being determinable by deducting from the quantitative total the quantitative determination on the direct positive reaction alone. Only a trace of crystalloid bilirubin suffices to give rise to a direct positive reaction, for they may frequently be encountered in sera with normal icterus indices and bilirubin content in lobar pneumonia, septicemia, multiple liver abscesses, cardiac failure, secondary syphilis, metastatic carcinoma of the liver, and following accidental or operative trauma.

The icterus index may be defined as a physical measurement of the yellow color intensity of serum, not directly proportional to the quantitative bilirubin content. Since bilirubin may occur in serum as a crystalloid, in true solution, or as a suspensoid colloid subject to variations in the size of the particles suspended, many of the well-known discrepancies between the icterus index and the total bilirubin content may be understood. A color producing substance in true solution might be expected to impart a greater color intensity to its solvent than an equal amount occurring in the medium as a suspended colloid. Hence, in a serum with a given icterus index much less crystalloid pigment need be present to cause that color intensity than would be required if it were in the colloidal state; therefore, it may be inferred that a serum yielding a relatively high bilirubin content contains a large part of the pigment in the colloidal state, and conversely, if the bilirubin content is relatively low, most of it is present in true solution. The icterus index, then, may be regarded as a function as well of the physical state of bilirubin as of its total quantity.

CORRELATION OF THE THREE TESTS

Hubbard has attempted to correlate the icterus index with the length of the delay period of the van den Bergh reaction, but found no definite point of demarcation in a large clinical series, except that as the index rose, the percentage of direct positive reactions increased and the shorter delay periods became more evident. Woodruff and his coworkers made some very interesting findings in a correlation of the icterus index and the quantitative bilirubin. To small amounts of normal dog serum they added known quantities of pure bilirubin, rendered soluble by alkali, and then, deducting the icterus index of the normal serum, determined the icterus index caused by the added bilirubin. On a graph with the known quantities of bilirubin as ordinates, the corresponding icterus indices were plotted in as the abscissae. The bilirubin range was 0-10 mg. per 100 c.c., and the icterus index range 0-100. Their "line of maximum density" bisected the graph diagonally from 0 to the icterus index 100 and the bilirubin 10, thus indicating that a quantitative bilirubin of 6.1 should give an index of 61, a bilirubin of 2.9 an index of 29, a relation expressed by a shift of the decimal point. There is shown, however, a striking characteristic in the variation of the points from the line of maximum density. Below the icterus index of approximately 16 practically all of the bilirubin values run above the line; between 16 and 30 there is evidence of a definite downward loop below the line; beyond 30 they vary widely on both sides of the line. It is this downward loop which commands attention, because it may be found clinically in exactly the same zone in lobar pneumonia in the white race, in pernicious anemia, familial jaundice, and in the newborn (placenta

blood). It has not as yet been observed in the black race in any of these entities, for it definitely does not occur in the ascent of the icterus in lobar pneumonia in the negro, and pernicious anemia and familial jaundice are so rare in the black race that none have as yet been studied.

The accompanying charts present the results of a correlation based on approximately 1700 sera examined by the three test method, from over 700 patients representing a wide variety of clinical entities. Since these tests were done by a single observer using a uniform technic, differences may be regarded as fairly valid in significance, with the factor of error a constant.

Chart 1 demonstrates the possible variation in total bilirubin content of a serum having an icterus index from the normal zone to 60. It is obvious that the icterus index fails to conform consistently with any constant total bilirubin content ratio.

There are no known cases of carotinemia in this group. The relatively high bilirubin values in and near the normal zone of the icterus index were found in sera from patients with familial jaundice and pernicious anemia, in placenta blood, and in sera from patients suffering only from trauma, accidental (contusions, fractures, deep lacerations, bullet wounds), or operative. In this traumatic group latent jaundice was a constant feature, often manifest for a short time in the sclerae, and evidently produced by the formation and release of bilirubin from hemoglobin in the interstitial blood extravasation at the site of the trauma. Direct positive van den Bergh reactions were very frequently encountered during the course of this icterus caused by trauma. The relatively low bilirubin values in the higher zones of the icterus index were found in serial studies as any jaundice was subsiding, occasionally in cirrhoses, and in negroes. If a line be drawn connecting the maximum bilirubin values in areas of high dot-density, two downward loops will be seen; a small one following the icterus index 10, and a very definite one between the index 16.6 and 30. These icterus indices, 10 and 16.6, have been found to be of extraordinary significance in a study of 224 cases of lobar pneumonia.

Several points are demonstrated on Chart 2. (1) For a given icterus index the bilirubin content is higher when the pigment is direct negative (or long delayed), and in the colloidal state, than when the pigment is direct positive and a crystalloid. (2) In sera with a direct negative (or long delayed) reaction a sudden increase in bilirubin content takes place when the icterus index arrives at 16.6. (3) As the icterus index passes 16.6, sera uniformly exhibited either a true direct positive reaction or an immediate golden accentuation on the addition of the diazo reagent. This icterus index seems to be the upper limit of color intensity which can be produced by colloidal bilirubin (serum hematoidin) before a change takes place in the van den Bergh reaction.

The data for the "dot-dash" line of direct negative bilirubin is derived from 200 sera from patients exhibiting icterus due to trauma, exclusive of those sera in which zonal positive or direct positive reactions were present; hence the use of the term "blood extravasations" in the legend.

On Chart 3 are plotted the average bilirubin contents for given icterus indices found in pernicious anemia (15 cases), familial jaundice (12 cases),

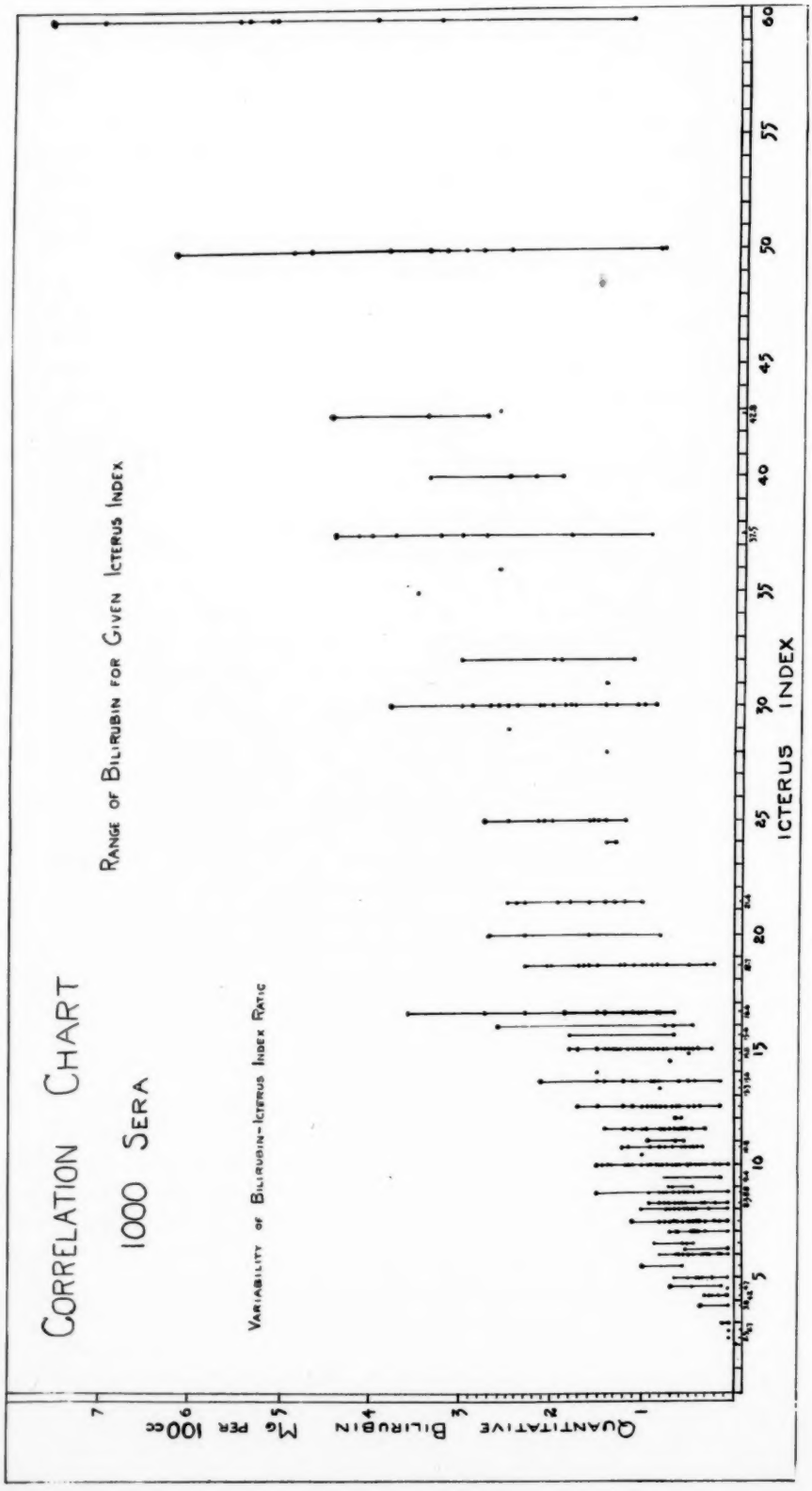

CORRELATION CHART
1000 SERA
RANGE OF BILIRUBIN FOR GIVEN ICTERUS INDEX
VARIABILITY OF BILIRUBIN-ICTERUS INDEX RATIO
QUANTITATIVE BILIRUBIN Mg PER 100cc
ICTERUS INDEX

Chart 1.

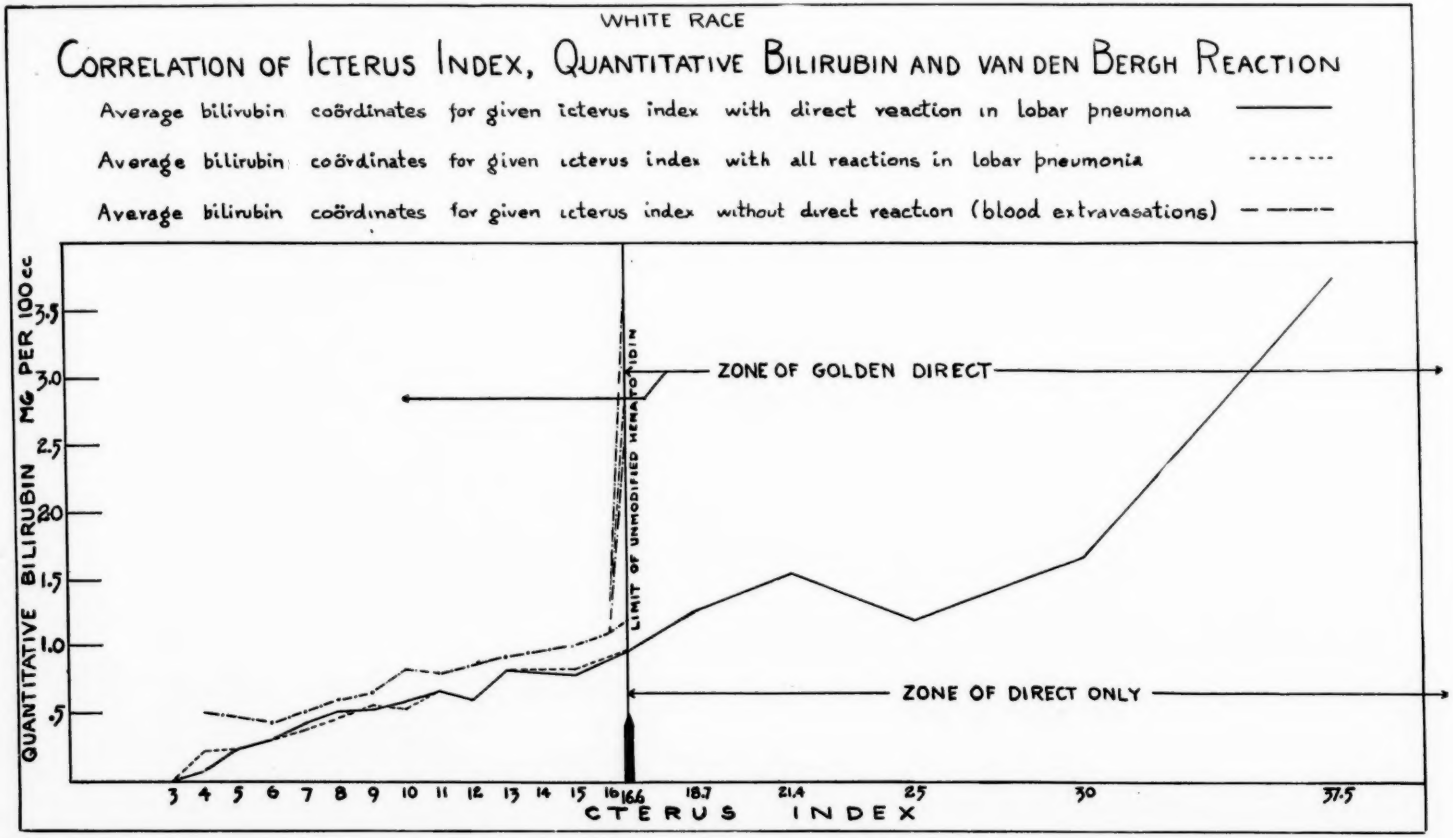

WHITE RACE
CORRELATION OF ICTERUS INDEX, QUANTITATIVE BILIRUBIN AND VAN DEN BERGH REACTION
Average bilirubin coördinates for given icterus index with direct reaction in lobar pneumonia
Average bilirubin coördinates for given icterus index with all reactions in lobar pneumonia
Average bilirubin coördinates for given icterus index without direct reaction (blood extravasations)
QUANTITATIVE BILIRUBIN MG PER 100 cc
3.5
3.0
2.5
2.0
1.5
1.0
.5
LIMIT OF UNMODIFIED HERATOIDIN
ZONE OF GOLDEN DIRECT
ZONE OF DIRECT ONLY
3 4 5 6 7 8 9 10 11 12 13 14 15 16 16.6 18.7 21.4 25 30 37.5
ICTERUS INDEX

Chart 2.

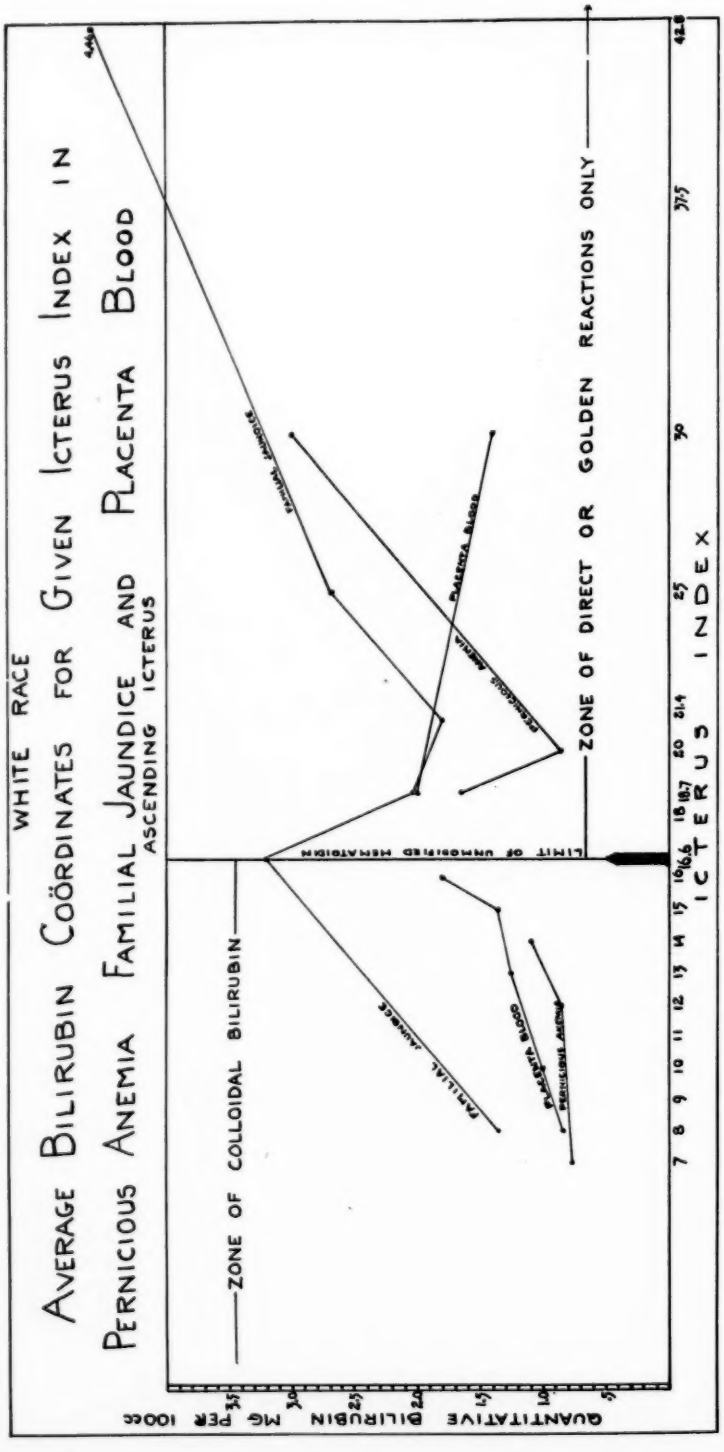

WHITE RACE
AVERAGE BILIRUBIN COÖRDINATES FOR GIVEN ICTERUS INDEX IN
PERNICIOUS ANEMIA FAMILIAL JAUNDICE AND PLACENTA BLOOD
ASCENDING ICTERUS
ZONE OF COLLOIDAL BILIRUBIN
LIMIT OF UNMODIFIED HEMATOIDIN
ZONE OF DIRECT OR GOLDEN REACTIONS ONLY
PERNICIOUS ANEMIA
PLACENTA BLOOD
FAMILIAL JAUNDICE
QUANTITATIVE BILIRUBIN MG PER 100cc
ICTERUS INDEX

Chart 3.

and the newborn (30 specimens of placenta blood). The downward loops in the bilirubin curves between the indices 16.6-30 are quite definitely indicated. In such entities direct positive reactions are rare, but were observed in one placenta blood (infant normal) and in two cases of pernicious anemia at indices 30 and 50.* Since this chart was made, two additional cases of familial

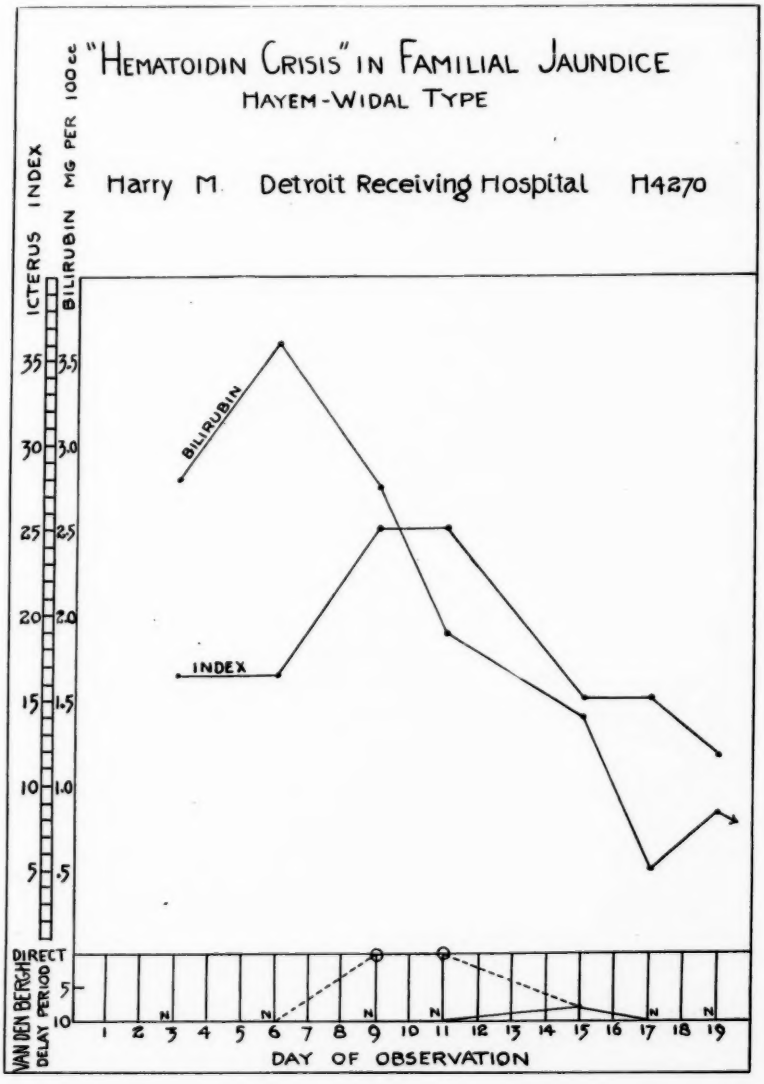


Chart 4.

jaundice have been observed, one exhibiting a direct positive reaction throughout the course of the icteric exacerbation, and the other, an exception to the previous findings, exhibiting icterus indices for five months in the 16.6 to 30 zone without evidence of either a downward loop or a golden reaction. The

*Greene has reported direct positive reactions in occasional cases of pernicious anemia and familial jaundice.

delay periods in the reactions, when not direct positive, were found to be shorter in placenta blood than in pernicious anemia, and longest in familial jaundice.

Chart 4 exhibits serially "the riddle of the icterus index 16.6" in a specific case of familial jaundice. The dotted line at the bottom of the chart where the van den Bergh reaction curve is plotted in terms of the length of the delay period, indicates by the circles on the *direct* line that with the rise of the icterus index from 16.6 to 25, accompanied by the decrease in serum bilirubin, the immediate golden reaction with the diazo reagent developed. The red direct reaction was consistently absent except for the development of an eight-minute delay period on the fifteenth day of observation as the accumulated bilirubin was passing through the liver parenchyma. The massing of direct negative bilirubin at the icterus index 16.6 without increasing the yellow intensity of the serum can be explained only by assuming the formation of larger colloidal aggregates of the pigment, unable to enter the liver parenchyma, terminated by a spontaneous physical change by which a crystalloid derivative was evolved, to which the liver was permeable, producing a greater color intensity in serum, although quantitatively less, because of greater solubility, as indicated by the immediate golden color change in the aqueous medium of the direct van den Bergh reaction.

TECHNIC OF TESTS

The following details of technic describe the uniform method used in this study. All three tests may quickly be done on each serum by the continuity of technic. They have been found adaptable only to a study of the changes in the icterus of a given clinical entity, and are usually of little or no value as an aid in differential diagnosis when used separately or in single determinations.

Icterus Index.—Using a Dubosecq colorimeter the serum from about 10 c.c. of fresh blood is compared directly with a standard 1-10,000 solution of potassium dichromate, which arbitrarily represents unity. The standard is usually set at 15 mm. The serum must be clear and yellow, free from hemolysis visible to the eye, turbidity, or murky brownish color. The comparison is best made using natural morning light from a window. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of serum}} = \text{icterus index}$$

The normal range is from 3 to 5, signifying that human serum has from 3 to 5 times the yellow color intensity of the arbitrary standard. There is no racial difference known. Latent jaundice may still be present even though the icterus index lies within normal limits, its detection then depending on the van den Bergh reaction and the quantitative bilirubin determination. Conversely, high icterus indices may not be indicative of abnormal bilirubin content when the serum color is other than clear and yellow. The possibility of an increase in luteins, lipochromes, and carotinoids must be considered, as well as, according to van den Bergh, hematin icterus.

Van den Bergh Reaction.—After determining the icterus index, 1 c.c. of serum is poured from the colorimeter cup into an accurately graduated centrifuge tube. The tube is slanted and from a pipette 0.5 c.c. of Ehrlich's diazo reagent (freshly mixed) is overlayed on the serum (technic originally used for urine). An immediate reddish, amber, or port-wine color reaction at the contact zone is indicative of a positive zonal reaction. Serum and reagent are then shaken and mixed so that a small amount of serum at the lower tip of the tube remains unmixed and can be used as a control of the color change above. The tube should

be watched for at least ten minutes for evidence of the beginning of a reddish, amber, or port-wine color change. If the color change occurs at once, it may be called direct positive. Should the reaction not begin until a few minutes have elapsed, the delay period should be stated as accurately as possible. When no change occurs up to ten minutes, the reaction may, for practical purposes, be called direct negative. If at the zone of contact or on mixing a sudden golden accentuation takes place, it may be called direct golden positive. These readings should also be made in natural daylight, preferably with a glazed glass window as a background. Normal sera are usually direct negative in the ten minute interval.

Most of the prevailing confusion in the interpretation of this test arises from the use of the term "biphasic," and from the fact that oxalated plasma evidently reacts differently from serum. The former frequently gives an immediate blue violet color,* while the latter practically never does, giving initially the reddish color alone when a direct positive reaction occurs. Hall has recommended that the term "biphasic" be discarded as meaningless, Andrewes disregards the quality of the color, and van den Bergh in 1928 reiterates, "no direct bluish color must appear." Hence, since the "direct prompt," or blue-violet, reaction does not exist when serum is used, and all direct positive reactions could be called "biphasic," there is no particular reason for retaining such a nomenclature.

The results of the van den Bergh reaction may be recorded as:

Direct positive
Direct delayed____minutes
Direct negative (10 minutes)

Positive zonal reactions may frequently be found when, after mixing, there seems to be no definite change up to two or three minutes. The golden accentuation should be recorded only when it can be demonstrated to a skeptic. It is often followed by a delayed red reaction.

Quantitative Bilirubin Determination.—After the determination of the van den Bergh reaction, add 2.5 c.c. of 95 per cent alcohol, then 1 c.c. of saturated ammonium sulphate, and place in the centrifuge for a few minutes. On removal from the centrifuge, three layers will be seen; an upper pink or ruby colored layer in which essentially all the bilirubin is contained in alcoholic solution as azo-bilirubin, a middle layer of compacted precipitated proteins, usually colorless, and a lower layer of ammonium sulphate solution. Determine from the graduations on the tube the volume in c.c. of the upper colored layer and pour off some of it into a colorimeter cup for comparison with van den Bergh's standard cobaltous sulphate solution, which represents the color intensity produced in a similar reaction by exactly 0.5 mg. of bilirubin per 100 c.c. For pale color reactions set the standard at 2 mm. in the colorimeter; for deep ruby reactions at 5 or 10 mm. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \frac{\text{Dilution factor}}{2} = \text{mg. bilirubin per 100 c.c. of serum}$$

By the use of the dilution factor the three tests may be run in continuity with as little as 0.4 c.c. of serum to work with, increasing somewhat, however, the factor of error.

$$\text{Dilution factor equals } \frac{\text{volume in c.c. of colored upper layer}}{\text{volume in c.c. of serum used}}$$

This quantitative determination (indirect reaction) is occasionally unsatisfactory due to anomalous factors in various sera. The alcoholic layer may not be comparable with the standard because of a yellow or orange color instead of the pink. A few drops of oxalic acid usually change the yellow color back to the pink, but a resulting turbidity often spoils the accuracy of comparison. In spite of the frequent difficulties encountered due to initial turbidity (removable by heating, by adding 1 to 2 drops of ether, or an additional c.c. of alcohol), and to the yellow anomaly, practical approximations can be made after the routine observation of a number of normal and icteric sera. With sera obviously deeply jaundiced little difficulty is encountered. Normal sera usually give either no grossly visible pink color in this reaction, or are but faintly reactive.

*The direct positive reaction on fresh bile is often blue-violet, but is usually red.

SUMMARY

Mann has shown that nascent bilirubin is produced extra-hepatically at a very rapid rate in the dog. The production rate in the human organism may be estimated as equally rapid, equivalent to approximately 14.3 mg. per 100 c.c. of serum volume in twenty-four hours. Mann found that such nascent bilirubin is direct negative in terms of the van den Bergh reaction. It has long been known that direct negative bilirubin is changed to the direct positive form by the liver parenchyma in mammals as it is excreted. The difference in these two forms of bilirubin has been found to depend on the physical state of the pigment: the direct negative form being free nascent bilirubin occurring as a suspensoid colloid; the direct positive form being an unstable crystalloid salt of bilirubin. Since the liver parenchyma is ordinarily impermeable to substances in the colloidal state, the conversion of free bilirubin to a crystalloid salt is a necessary element of the excretion process.

In consideration of the relatively massive normal daily production of bilirubin, it is obvious that the excretion of the pigment by the liver must at all times be efficiently maintained to preserve the balance and prevent a constantly ascending icterus. From the experiments of Naunyn, Minkowski, and McNee it appears plausible that the Küpffer cells in mammals act as the acceptors of nascent colloidal bilirubin as their function in the excretion of the pigment, their relative permeability to the pigment being the threshold determinant under normal conditions and in functional icterus.

The direct delayed van den Bergh reaction can be interpreted in terms of the length of its delay period as indicating intermediate stages in the transition of nascent bilirubin to the crystalloid form, arising from pigmentary congestion in the liver parenchyma, the speed of the reaction depending on the relative solubility of the conversion products. Minute quantities of fully converted crystalloid bilirubin give rise to direct positive reactions in serum, and when both forms exist together, the reaction is direct positive.

The icterus index may be better understood as a "yellow intensity" index and not directly proportional to the bilirubin content, because of the many possible differences in the physical state and solubility of the pigment present.

In correlations of the results from over 1700 sera examined by the three test method, it has been found that: (1) The icterus index fails to conform consistently with any constant proportion of total bilirubin. (2) For a given icterus index the bilirubin content of the serum is higher when it exists in the colloidal state than when it is crystalloidal. (3) Colloidal bilirubin accumulates in the blood stream at the icterus index 16.6, but fails to impart a higher color intensity to the serum in which it is suspended until it undergoes a physical change, expressed by the development of an immediate type of van den Bergh reaction. (4) In sera from the newborn, pernicious anemia, and familial jaundice, a downward loop in total bilirubin content occurs as the icterus index rises from 16.6 to 30, accompanied by the appearance of an anomalous immediate golden accentuation in the direct van den Bergh reaction. One exception to statements 3 and 4 is cited.

A uniform technic for conducting the three tests in continuity on small amounts of serum is outlined, in which the innovation of overlaying the diazo reagent on the serum prior to mixing is proposed as a more sensitive method to detect the presence of crystalloid bilirubin. The terminology of Dr. A. A. Hijmans van den Bergh in the interpretation of the results of the reaction, as well as in the classification of jaundice as obstructive or functional, has proved most adaptable to the understanding of the pathologic physiology of icterus in the light of recent experimental work.

The results as presented in this paper comprise a part of an intensive clinical study of jaundice based on the serial application of the three tests for serum bilirubin, in which to date over 1700 sera from more than 700 cases have been studied, representing probably most of the conditions in which latent or clinical jaundice exists. Much of this work was done in the Department of Pathology at the Detroit College of Medicine and Surgery, of which Professor James E. Davis is the head, during the years 1929 and 1930.

REFERENCES

Andrewes, C. H.: Nature of the Difference Between the Bilirubins of Obstructive and Hemolytic Jaundice, Brit. J. Exper. Path. **5:** 213, 1924.

Aschoff, L.: Theses and Antitheses in Theory of Bilirubin Formation, Klin. Wehnschr. **5:** 1260, 1926.

van den Bergh, A. A. Hijmans: Der Gallenfarbstoff im Blute, 1928 re-issue.

von Bergmann, G.: Functional Pathology of the Liver, Klin. Wehnschr. **6:** 776, 1927.

Bernheim, Alice R.: The Icterus Index: a Quantitative Estimation of Bilirubinemia, J. A. M. A. **82:** 291, 1924. Bilirubinemia: Significance of Variations, Arch. Path. **58:** 747, 1926.

Blankenhorn, M. A.: Acholuric Jaundice, Arch. Int. Med. **27:** 131, 1921.

Bollman, J. L., and Mann, F. C.: The van den Bergh Reaction in Jaundice Following Complete Removal of the Liver, Proc. Staff Meet., May Clinic **4:** 261, 1929.

Burton, E. F.: The Physical Properties of Colloidal Solutions, ed. 2, Monographs on Physics, 1921, Longmans, Green & Co.

Collinson, C. A., and Fowweather, F. S.: The Two Forms of Bilirubin Demonstrated by the van den Bergh Reaction, Brit. Med. J. **1:** 1081, 1926.

Cutten, Carrie, Emerson, Edith E., and Woodruff, Warriner: Icterus Index: Spectrophotometric and Quantitative Studies, Arch. Int. Med. **41:** 428, 1928.

Diamond, J. S.: Value of Routine Estimations of Blood Bilirubin, Am. J. Med. Sc. **176:** 321, 1928.

Greene, C. H., and Conner, H. M.: Comparative Study of Tests for Hepatic Function in Certain Diseases of the Hematopoietic System, Arch. Int. Med. **38:** 167, 1926.

Hall, W. W.: The van den Bergh Reaction for Serum Bilirubin With Notes on Interpretation of Technic, J. Lab. & Clin. Med. **12:** 529, 1927.

Hoover, C. F., and Blankenhorn, M. A.: Dissociated Jaundice, Arch. Int. Med. **18:** 289, 1916.

Hubbard, R. S., and Allison, C. B.: Comparison of the Icterus Index and Direct van den Bergh Tests, Proc. Soc. Exper. Biol. & Med. **26:** 438, 1929.

Hunter, G.: Two Types of Bilirubin Diazo Reaction in Serum: Hypothesis on Nature of Bilirubin in Serum from Hemolytic Jaundice, Brit. J. Exper. Path. **11:** 415, 1930.

Kanner, O.: Biliary Pigmentation of Küpffer Cells in Different Forms of Jaundice, Compt. rend. Soc. de Biol. **95:** 1311, 1926.

M'Gowan, J. P.: Some Aspects of the van den Bergh Test for Bilirubin, Ed. Med. J. **36:** 242, 1929. The Alkali Reserve of the Blood in Relation to the van den Bergh Bilirubin Test, Ed. Med. J. **37:** 28, 1930.

McNee, J. W.: Jaundice: A Review of Recent Work, Quart. J. Med. **16:** 390, 1923.

Mann, F. C.: Site of Formation and Source of Bilirubin, Arch. Path. **2:** 516, 1926. Abstract of Discussion on Papers of Drs. Judd, Marshall, Payne, Blankenhorn, and Ivy: 81st Annual Session of American Medical Association, J. A. M. A. **95:** 1072, 1930.

Perkin, F. S.: Blood Bilirubin: Estimation and Clinical Significance, Arch. Int. Med. **40:** 195, 1927.

Rich, A. R.: Extra-hepatic Formation of Bile Pigment, Johns Hopkins Hosp. Bull. **36:** 233, 1925.

Thannhauser, J. S., and Anderson, E.: Bilirubin in Blood Serum, Deutsche. Arch. f. klin. med. **137:** 179, 1921.

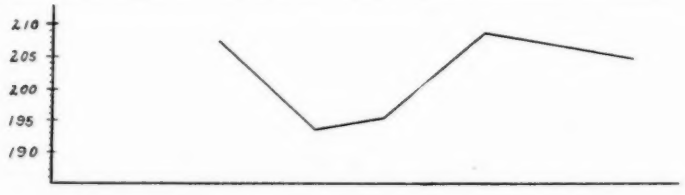
THE CATAMENIA AND OXYGEN CONSUMPTION*

By Charles L. Wible, Lincoln, Neb.

ZUNTZ[1] in 1906 concluded that the intensity of oxidation processes in women changed very little during menstruation. Gephart and DuBois[2] (1916) confirmed these findings. Snell, Ford and Rowntree[3] (1920) found a rise just before and during the menses, with a marked fall after the period. Rowe and Eakin[4] (1921) reported a rise of 13 to 18 per cent above normal previous to menstruation and a low rate during the period and for approximately two weeks after menstruation. Wiltshire[5] (1921) and very shortly

Table I

Subject	No. of menstrual cycles	Consumption of Oxygen (c.c. per minute)				
		1-4 days before menstruation	During menstruation		5-8 days after end of menstruation	2 weeks after end of menstruation
			1st	2nd		
G. W. II	1	210	189	200	209	206
	2	208	189	202	210	198
	3	201	192	180	197	199
	4	208	184	198	200	201
	5	211	200	209	213	210
	6	205	180	200	207	205
	7	211	203	202	205	208
	8	210	206	205	214	212
	9	208	199	179	207	204
	10	204	188	206	211	210
	11	195	195	190	200	201
	12	217	199	180	217	209
	Average	207	193	195	208	205



after Blunt and Dye[6] found no elevation in the basal metabolism of women before and during the menses. Asher[7] (1920) stated that "a direct influence of the sexual organs on metabolism does not exist." It is also stated by Geist and Goldberger[8] that castration in women with previously functioning ovaries has no effect on basal metabolism. Wakeham[9] (1923) concludes that there is a distinct fall in basal metabolic rate during or immediately after menstruation which is preceded by a rise. Benedict and Finn[10] (1928) after extensive investigation of the problem reported that the oxygen consumption was lowest during the menstrual period and highest about one week after menstruation ceased. Griffith[11] (1929) found that menstruation lowered general metabolism.

*From the Department of Physiology and Pharmacology, University of Nebraska.
Received for publication, November 10, 1930.

Women students are continually referred to our Student Health Dispensary for metabolism tests. Such conflicting reports as are above cited upon the metabolism of women during menstruation caused us to avoid the taking of such tests near the catamenia and also suggested a study of the problem. This study was initiated in September, 1927.

Fortunate conditions for such a study exist in our Department of Physiology throughout the school year. The curriculum carries a course in Sex Hygiene for women students. The enrollment is always large. A number of these young women volunteered for the study and took considerable interest in our findings. In addition to this group several special students from our own department were available for the experiment over the entire period September, 1927, to September, 1929.

TABLE II

SUBJECT	NO. OF MENSTRUAL CYCLES COVERED BY EXPERIMENT	AVERAGE CONSUMPTION OF OXYGEN (C.C. PER MINUTE)				
		1-4 DAYS BEFORE MENSTRUATION	DURING MENSTRUATION 1ST	DURING MENSTRUATION 2ND	5-8 DAYS AFTER END OF MENSTRUATION	2 WEEKS AFTER END OF MENSTRUATION
I. M. O.	7	201	191	189	199	201
II. G. W.	12	207	193	195	208	205
III. H. M.	5	197	183	188	197	200
IV. B. B.	5	185	181	175	183	187
V. L. H.	8	195	195	188	198	197
VI. B. M.	3	200	199	187	194	201
VII. N. A.	5	196	186	190	193	197
VIII. A. C.	5	189	187	183	195	193
IX. A. K.	7	190	188	187	192	192
X. M. E.	5	180	177	176	180	193
XI. E. F.	5	188	182	181	196	186
XII. B. J.	5	180	173	175	178	189
XIII. M. T.	5	191	188	190	192	190
XIV. O. L.	6	207	197	187	205	206
XV. A. P.	4	198	196	191	199	201
XVI. A. O.	3	187	187	183	196	197
XVII. L. C.	5	197	188	186	200	199
XVIII. C. H.	4	188	172	176	181	191
XIX. L. P.	3	185	190	191	182	182
XX. P. M.	4	186	177	178	189	193
XXI. L. K.	8	206	198	201	205	206
XXII. V. H.	4	196	186	185	198	199

The closed circuit oxygen consumption type of apparatus was utilized in this work. Routine records of buccal temperature, room temperature, pulse rate, and barometric pressure were taken. All tests were made in the reclining position and in the early morning, fourteen to sixteen hours after the previous night's meal. The room temperature was maintained at a comfortable level with plenty of ventilation. Every subject was allowed a rest period of thirty minutes before beginning the test. Individuals with an indication of any circulatory deficiency were excluded from the experiment. Every step was taken to insure comfort for the subjects during the tests.

A test for leakage in the apparatus preceded every determination. To insure against any possibility of nervousness in connection with the first test of a series, each subject was allowed preliminary trials.

Tests were made on 22 different subjects covering a total of 118 menstrual cycles. Each cycle involved 5 tests distributed as follows: One to four days before the menstruation, two tests during menstruation, the first one usually falling on the first or second day; five to eight days after the cessation of menstruation and lastly, two weeks after cessation of menstruation.

The figures shown in Table I are derived from subject No. II (G. W.). The curve accompanying Table I is the result of the plotting of averages. This curve represents in general the type of curve secured for each subject.

The average consumption of oxygen for each subject during each period of the experiment is given in Table II. It will be noted that with one exception, subject XIX, these results indicate a low oxygen consumption during menstruation. About one-half of the subjects showed one or two menstrual periods in which oxygen consumption during menstruation had a tendency to rise above the premenstrual consumption. These, however, were much in minority and the figures were in nearly every case lower than the consumption five to eight days after cessation of menstruation. The values secured do not indicate a premenstrual rise. The period of highest oxygen consumption seems to be two weeks after cessation of menstruation.

REFERENCES

1. Zuntz, L.: Uber die menstruelle Wellenbewegung der weiblichen Lebensprozesse, Arch. f. Physiol. pp. 393-396, 1906.
2. Gephart, F. C., and DuBois, E. F.: The Basal Metabolism of Normal Adults With Special Reference to Surface Area, Arch. Int. Med. **17:** 902, 1916.
3. Snell, A. M., Ford, F., and Rowntree, L. G.: Studies in Basal Metabolism, J. A. M. A. **75:** 515, 1920.
4. Rowe, A. H., and Eakin, M.: Monthly Fluctuations in the Normal Metabolic Rates of Men and Women, Calif. State Med. J. **19:** 320, 1921.
5. Wiltshire, M. O. P.: Basal Metabolism in Menstruation, Lancet **2:** 388, 1921.
6. Blunt, K., and Dye, M.: Basal Metabolism of Normal Women, J. Biol. Chem. **47:** 69, 1921.
7. Asher, L., and Bertschi, B.: Beitrage zur physiologie der Drusen (Asher), Untersuchungen uber den respiratorischen Stoffwechsel kastrierter Kaninchen (Bertschi), Biochem. Ztschr. **106:** 37, 1920.
8. Geist, S. H., and Goldberger, M. A.: A Study of the Basal Metabolism, Weight, and Blood Chemistry Following Bilateral Oophorectomy, Am. J. Obst. & Gynec. **12:** 206, 1926.
9. Wakeham, W.: Basal Metabolism and the Menstrual Cycle, J. Biol. Chem. **56:** 555, 1923.
10. Benedict, F. G., and Finn, M.D.: Normal Menstruation and Gaseous Metabolism, Am. J. Physiol. **86:** 1, 1928.
11. Griffith, F. R., and others: The Metabolism and Body Temperature (Oral) Under Basal Conditions, Am. J. Physiol. **87:** 602, 1929.

STUDY ON A SERIES OF ARTHRITIC PATIENTS UNDER CONTINUOUS MONO-IODO-CINCHOPHEN TREATMENT WITH SPECIAL REFERENCE TO THE ACTION OF THE CINCHOPHEN MOLECULE ON THE LIVER TRACT*

By E. P. Corson White, B.A., M.D., Philadelphia, Pa.

SINCE 1923, when Worster-Drought[1] described the first case of cinchophen toxicity, a number of more or less carefully studied cases have been reported in the literature. These have been due not only to cinchophen itself but to its combinations and derivatives. The symptoms are always that of a moderate or severe toxic jaundice, which often results in the death of the individual. The autopsy shows acute yellow atrophy of the liver with its associated pathology.

In the history of these cases it is impressive to note that the presence of symptoms of poisoning, or the severity of the symptoms, is not proportional to the length of time the drug has been taken or to the amount of the drug ingested. There are records of an almost daily consumption over periods of years, with no bad effects. This is further substantiated by the relatively small number of proved cases of yellow atrophy in relation to the amount of cinchophen and derivatives annually consumed in the United States (approximately 100,000 pounds per year).

The one patient, Case 22, responsible for this study has taken cinchophen almost continuously for six years. Poisoning also has occurred a week or weeks after discontinuing the administration. These facts indicate clearly that the responsible factor must be in the individual taking the drug.

Chemically, cinchophen is a phenylquinoline carboxylic acid. While the fate of these compounds in the system has been studied in the past, these investigations have thrown little light on the nature of the decomposition products and their elimination.[2, 3, 4] However, it is reasonable to believe that the liver plays a part in the decomposition of these complex products with the formation of secondary products of a more or less specific toxic action on this organ.

It, therefore, seemed to us to be of value, before instituting medication with cinchophen preparations, to examine the patients carefully for any evidence of liver or possible pancreatic dysfunction.

In this study a group of arthritis cases, that under the previous treatment had failed to respond, were set aside for treatment with mono-iodo-cinchophen.

In all a history and physical examination were obtained with extreme care, making especial effort to locate the original foci, so as to exclude any and all cases showing factors suggestive of disease or inefficiency of these or-

*From the Laboratories of the Orthopedic Hospital.
Received for publication, April 30, 1931.

gans. The majority of the patients were drawn from the clinic service of Dr. W. J. Taylor and the laboratory studies were made in the laboratories of the Orthopedic Hospital, Philadelphia, Pa.

TABLE I

CASES	1	2	3	4	5
Diagnosis	Severe osteo-arthritis deformans	Chronic infectious	Chronic infectious	Chronic hypertrophic	Arthritis deformans
Involvement	General	Hands, knees	Hands, knees, elbows	Knees	General
Age	63 years	58 years	54 years	62 years	32 years
Duration	9 years	22 years	3 years	4 years	3 years
Foci	Tonsils, teeth	Colon	Colon	Tonsils	Not found
Treatment period	17 weeks	19 weeks	19 weeks	19 weeks	17 weeks
W. R.	Neg.	Neg.	Neg.	Neg.	Neg.
R. B. C.*	3880000	4050000	4080000	4180000	4020000
	4610000	4420000	4100000	4080000	4080000
W. B. C.	5800	6100	5800	6500	6700
	8000	7600	7800	6800	7000
Hb	64	79	78	83	79
	74	80	80	80	80
N. P. N.	28.4	30.1	26.2	26.4	27.8
	28.2	30	26.1	27	28.7
Urea	13.4	15.1	12.8	13.2	13.8
	13.6	15.1	13.1	13.7	14
Uric Acid	2.1	2.8	2.7	2.3	2.7
	2.2	2.4	2.8	2.9	2.4
Creatinin	1.6	1.8	1.5	1.5	1.7
	1.6	1.6	1.7	1.9	1.3
Glucose	84	91.3	99.4	101.3	84.1
	91.1	88.7	101.2	98.6	84
Glucose tolerance	N	N	N	N	N
	N	N	N	N	N
Levulose tolerance	N	N	N	N	N
	N	N	N	N	N
Epinephrine response	Neg.	Neg.	Neg.	Neg.	Neg.
	Neg.	Neg.	Neg.	Neg.	Neg.
Icteric index	4.1	4.5	4	4	4
	3.9	4.4	3.7	4.1	4
Van den Bergh	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
Rosenthal test	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	2% 15′ 0 – 1 hr.
	2.5% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.	2% 15′ 0 – 1 hr.
Bile drainage	Neg.	Not obtained	Not obtained	Neg.	Not obtained
	Neg.	Not obtained	Negative	Neg.	Not obtained
Urobilogen	Neg.	Neg.	Neg.	Neg.	Neg.
	Neg.	Neg.	Neg.	Neg.	Neg.
Urine findings	Neg.	Neg.	Alb. neg. casts	Neg.	Neg.

*In each case the first line represents laboratory findings before treatment, the second line represents laboratory findings after treatment.

N—indicates Normal. Neg.—indicates Negative.

Twenty-one cases were accepted as being entirely free from any symptoms or signs of liver, duodenal or pancreatic disease, as far as could be determined.

The laboratory examination consisted in Wassermann tests, blood counts and differential blood chemistry, nonprotein nitrogen, uric acid, creatinine, glucose and levulose-sugar tolerances and epinephrine response, van den Bergh, icteric index and Rosenthal tests and, where possible, a bile drainage.

These patients were then put on mono-iodo-cinchophen two capsules three times daily. Five had the drug for seventeen weeks, four for nineteen weeks, six for twenty weeks, two for twenty-four weeks, and four for twenty-eight weeks. The average length of uninterrupted administration totaled twenty-

TABLE I—CONT'D

6	7	8	9	10	11
Arthritis deformans	Chronic hypertrophic	Arthritis deformans	Chronic hypertrophic	Chronic atrophic	Chronic atrophic
General	Knees, wrists, spine	General	Hips, knees	Elbows, fingers	Knees
38 years	58 years	44 years	47 years	69 years	58 years
7 years	3 years	29 years	3 years	14 months	4 years
Tonsils, teeth	Teeth	Teeth, tonsils	Colon	Teeth, tonsils	Sinuses, mastoid
17 weeks	17 weeks	20 weeks	20 weeks	19 weeks	20 weeks
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
3950000	3970000	4000000	3700000	3780000	4000000
3930000	4020000	4090000	4110000	3760000	4390000
7100	8200	11500	8100	8000	7900
8600	7600	9100	8800	8100	7900
74	70	78	74	74	79
76	78	80	75	74	81
28.1	31	24.7	28	26.3	27
28.1	30.1	24.1	28.1	27	30
14	15.6	12.3	14.2	12.9	13.8
14.1	15	12	14.2	13.1	14.8
2.4	2.9	2.2	2.1	2.7	3.1
2.2	2.4	2.5	2.1	2.6	2.9
1.9	2	1.8	1.4	2.0	1.9
1.1	1.5	1.9	1.7	1.8	2.1
88.8	108.2	112.2	102.4	99.7	98.3
92.3	104.1	109.2	106.1	101.4	110.4
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
3.8	3.8	3.8	4.0	3.7	4.1
3.9	4.1	3.9	3.9	3.9	4.0
D– ID–	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
D– ID–	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
5% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.
4% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.
Not obtained	Not obtained	Neg.	Neg.	Neg.	Neg.
Not obtained	Not obtained	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Alb. neg., casts, pus cells	Neg.	Alb. casts pus cells	Neg.	Neg.	Neg.

one weeks. Some of these laboratory tests were carried out every week in order to catch any evidence of beginning trouble and all were repeated on the day on which the drug was discontinued and again one week and two weeks after the final withdrawal.

SUMMARY

Table I shows the results: (1) of the examinations made before institution of treatment by mono-iodo-cinchophen and (2) of the examinations made after withdrawal of the drug. At no time during the observation period did any of these patients show any variation from the normal.

TABLE I—CONT'D

CASES	12	13	14	15	16
Diagnosis	Senile atrophic	Arthritis deformans	Arthritis deformans	Arthritis deformans	Chronic hypertrophic
Involvement	Left hip	General	General	General	Sacro-iliac, left ankle
Age	73 years	57 years	53 years	42 years	28 years
Duration	2 years	21 years	17 years	12 years	9 months
Foci	Colon	Teeth, tonsils	Tonsils, ethmoid	Teeth	Colon
Treatment period	17 weeks	24 weeks	28 weeks	28 weeks	24 weeks
W. R.	Neg.	Neg.	Neg.	Neg.	Neg.
R. B. C.*	3800000	4010000	4180000	3060000	4210000
	4680000	4350000	4270000	3350000	4480000
W. B. C.	7800	6900	8100	7500	7600
	8400	8100	8600	8000	7800
Hb	75	78	78	60	74
	83	85	77	64	74
N. P. N.	27.8	28.6	30.2	28.2	26.4
	26.8	28.8	28.2	29.7	30
Urea	13.7	14.4	14.4	13.4	13.2
	13.6	14.2	13.9	14.1	14.9
Uric Acid	2.1	2.4	2.3	2.7	2.8
	2.9	2.5	2.7	2.2	2.7
Creatinin	2.2	1.9	1.9	1.8	2
	1.9	2.1	1.8	1.9	1
Glucose	89.2	99.4	114.7	118.3	98.9
	92.4	107.2	110.1	100.6	107.5
Glucose tolerance	N	N	N	N	N
	N	N	N	N	N
Levulose tolerance	N	N	N	N	N
	N	N	N	N	N
Epinephrine response	Neg.	Neg.	Neg.	Neg.	Neg.
	Neg.	Neg.	Neg.	Neg.	Neg.
Icteric index	3.8	4.0	4.1	4.0	3.9
	4.0	4.2	4.5	4.4	4.0
Van den Bergh	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
Rosenthal test	2% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.	2% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.
	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	2% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.
Bile drainage	Neg.	Neg.	Neg.	Neg.	
	Neg.	Neg.	Neg.	Neg.	Neg.
Urobilogen	Neg.	Neg.	Neg.	Neg.	Neg.
	Neg.	Neg.	Neg.	Neg.	Neg.
Urine findings	Indican	Neg.	Pus cells	Neg.	Neg.

All of the 21 patients recorded were advanced cases of arthritis with multiple involvements but were a group apparently without any lesions of the upper intestinal area. All took the drug without undue symptoms, some with improvement, some without, and this factor had apparently no influence on the findings. The drug was administered uninterruptedly in order that the results might be compared with cases of self-medication so common in the field of the arthritics.

Case 22 represents the original subject, self-treated for a period of considerably over six years. Two series of tests were made at an interval of six months.

While this group of cases is comparatively small, the length of time given over to the uninterrupted use of the drug is long, averaging five months, they are reported:

TABLE I—CONT'D

17	18	19	20	21	22
Arthritis deformans	Atrophic	Hypertrophic	Multiple infectious	Hypertrophic	Arthritis deformans
General	Hip	Shoulders, knees	Elbows, hands, knees, ankles	Lumbosacro iliac, elbows	General
67 years	58 years	42 years	23 years	39 years	52 years
28 years	8 years	3 years	9 months	4 years	17 years
Sinuses, ehtmoid	Sinus, ethmoid, sphenoid	Colon	Teeth, tonsils	?	?
28 weeks	20 weeks	20 weeks	28 weeks	20 weeks	6 years, 9 mo. (Cinchophen)
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
4050000	4210000	3710000	3650000	4130000	4010000
4110000	4180000	4220000	4260000	4180000	4090000
8200	8000	8000	6500	11200	7200
8100	8800	8900	8100	9100	7600
75	80	75	73	80	80
74	80	84	80	80	79
26.8	29.3	27.2	30	30	26.3
26.6	28.1	27.4	30.1	29.4	26.5
13.4	15	14.1	14.9	14.8	13.1
13.4	14.3	13.8	14.9	13.9	13.3
3.1	2.7	3.3	3.1	2.9	2.9
2.8	2.8	3.0	2.7	2.9	3.0
1.9	1.2	1.1	1.8	1.9	1.5
1.8	1.6	1.7	1.9	2.0	1.4
86.1	94.3	96.2	107.3	108.8	111.3
85.7	99.5	99.4	99.9	110.3	101.7
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
4.0	3.5	3.9	4	3.9	4.1
4.1	3.3	3.7	3.9	3.9	4.0
D– ID–	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
D– ID–	D– ID–	D– ID–	D– ID–	D– ID–	D– ID–
3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.
3% 15′ 0 – 1 hr.	3% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.	5% 15′ 0 – 1 hr.	4% 15′ 0 – 1 hr.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Alb., pus cells	Alb., pus cells, casts	Indican	Neg.	Neg.	Alb., casts

1. As a method of possible safeguarding those persons using these drugs.

2. As an effort to determine a reason for the apparent inconsistency in the appearance of toxic reactions from cinchopen and its congeners.

In the one fatal case of cinchopen poisoning previously studied by the author, there was a history of symptoms suggestive of liver and gall bladder disease antedating the taking of cinchophen by at least four years.

REFERENCES

1. Worster-Drought, C. C.: Atophan Poisoning, Brit. M. J. **1:** 148, 1923.
2. Rotter, L.: Atophan and Several of Its Derivatives, Ztschr. Exper. Path. Therap. **19:** 176, 1918.
3. Willcox, W. H.: Atophan Derivatives in Rheumatism, Brit. M. J. **2:** 273, 1926.
4. Sutton, D. C.: Acute Yellow Atrophy of the Liver Following the Taking of Cinchophen, J. A. M. A. **91:** 310, 1928.

DIABETES MELLITUS AND THE GASTRIC SECRETION*†

By Michael G. Wohl, M.D., Philadelphia, Pa.

IN THE treatment of patients with diabetes mellitus there has been insufficient stress laid on the gastrointestinal tract. The high grade spastic obstipation is frequently commented upon; however, the not uncommon gastric complaints, such as anorexia, feeling of distention, localizing discomfort after meals, and inability to consume the allotted amount of food have not attracted as much attention as they deserve.

Of 110 patients with diabetes mellitus, studied at the Mercy Hospital, Council Bluffs, Iowa, and at the Temple University Hospital, Philadelphia, 19 patients presented symptoms related to a disturbed gastrointestinal function.

The gastric contents were studied by the fractional method in 33 cases, 19 of whom manifested some gastrointestinal symptoms. Roentgen examinations were made in 12 patients and in 24 the stool was examined. In 10 the duodenal contents were obtained by means of the duodenal tube, and studied for the presence of pancreatic ferments.

At present I shall confine myself to the consideration of the gastric acidity. The group of patients comprised 21 females and 12 males.

The amount of sugar in the fasting blood varied from 160 mg. per 100 c.c. of blood (the lowest) to 380 mg. (the highest). Eighteen patients out of the 33 showed a hypoacidity and achlorhydria. Eleven showed achlorhydria and seven hypoacidity. Four patients had a hyperacidity. Eleven showed an acidity within the normal range. Two out of the hyperacidity group complained of a burning sensation after meals and of a marked constipation. Four out of the normal group had some gastrointestinal complaint. The patients were placed on proper diabetic diets, and insulin was administered when diet alone did not suffice to reduce the blood sugar to a level of 120 to 130 mg. per 100 c.c. and when the urine still showed sugar. The patients with hypoacidity and achlorhydria were given, in addition, dilute hydrochloric acid with each meal (5 c.c.). The gastric symptoms disappeared in 11 patients. Before the administration of dilute hydrochloric acid, they exhibited some gastric symptoms. Two had chronic cholecystitis, one of whom was operated upon, and improved; one has not improved. (Table I includes a summary of these patients with achlorhydria and hypoacidity.)

That the symptoms might be attributed to the achlorhydria can be deduced from the fact that no gastrointestinal pathology was demonstrated by the roentgen ray in 10 patients; their gastric complaints disappeared or dimin-

*From the Department of Internal Medicine, Temple University Medical School, Philadelphia, Pa.

Received for publication, April 14, 1931.

†This study was commenced at the Mercy Hospital, Council Bluffs, Iowa, in 1925; since 1929 it has been continued at Temple University Hospital, Philadelphia, Pa.

ished after the administration of the dilute hydrochloric acid. I am aware of the fact, however, that achlorhydria and hypoacidity existed in five of the patients without any gastric symptoms.

TABLE I

CASES OF DIABETES SHOWING ACHLORHYDRIA AND HYPOACIDITY

SEX	AGE	PROBABLE DURATION OF DIABETES	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR PER CENT	ACETONE AND DIACETIC	GASTRIC ANALYSIS
♀	50	6 yr.	230	2.0	–	Free HCl ------ 0 Total acid max.- 10
♂	40	3 yr.	190	1.6	++	Free HCl ------ 0 Total acid max.- 14
♀	45	4 yr.	200	1.4	–	Free HCl ------ 0 Total acid max.- 15
♀	48	6 yr.	200	1.6	–	Free HCl ------ 0 Total acid max.- 5
♀	50	8 yr.	190	1.4	–	Free HCl ------ 0 Total acid max.- 17
♀	55	8 yr.	220	2.5	–	Free HCl ------ 0 Total acid max.- 15
♀	48	12 yr.	210	1.8	++	Free HCl ------ 0 Total acid max.- 38
♀	46	7 yr.	180	0.3	–	Free HCl ------ 0 Total acid max.- 25
♂	65	15 yr.	225	2.2	–	Free HCl ------ 0 Total acid max.- 35
♀	54	12 yr.	200	1.8	–	Free HCl ------ 0 Total acid max.- 30
♂	50	11 yr.	200	1.6	–	Free HCl ------ 0 Total acid max.- 30
♂	50	8 yr.	200	1.8	–	Free HCl ------ 5 Total acid max.- 50
♂	46	3 yr.	200	1.4	–	Free HCl max.- 5 Total acid max.- 30
♂	57	7 yr.	280	2.4	+	Free HCl max.- 18 Total acid max.- 45
♀	35	2 yr.	190	1.2	–	Free HCl max.- 15 Total acid max.- 38
♂	46	9 yr.	210	2.3	–	Free HCl max.- 12 Total acid max.- 45
♀	58	11 yr.	190	1.4	–	Free HCl max.- 6 Total acid max.- 20
♀	52	13 yr.	185	1.2	–	Free HCl max.- 12 Total acid max. 48

Knowledge of the gastric acidity in patients with diabetes mellitus may be of value in the interpretation of metabolism of carbohydrates. Glucose metabolism depends upon the activity of several factors. Among these, absorption (intestine), storage (liver), and utilization (tissues) (insulin is only one link in the chain) are of prime importance. Whether the rapid emptying of the stomach due to achlorhydria permits an unequal absorption from the intestine[1] or some other obscure change incident to achlorhydria influences the fluctuation in blood and urine sugar is difficult to state at the present.

It is a clinical observation, however, that an individual who suffers from a functional hyperchlorhydria, begins to show sugar in the urine once an achlorhydria develops. Explanations concerning the functional activity on the part of digestive organs and the blood-sugar concentration are not lack-

ing. We owe to Cammidge and Howard[2] the credit for focusing our attention on the fact that the functional disturbances of gastric secretion play a part in carbohydrate metabolism.

They are of the opinion that the alkaline secretion (pancreatic) below the pylorus which is initiated by the gastric hydrochloric acid causes a relative acidosis; they ascribe this to the abstraction of the bases from the blood for the formation of pancreatic secretion. They have also shown that the entrance of alkaline bases into the circulation is accompanied by a lowering of the blood-sugar level, and vice versa an increase in the fixed acid of the blood causes a rise in the blood sugar. The intimate relation between the acid base equilibrium and some phases of carbohydrate metabolism finds a measure of corroboration in the frequency with which hyperglycemia and glycosuria are worse in the early morning hours in some patients with diabetes. Thus Leathes[3] finds a high CO_2 of the alveolar air immediately upon waking, due to the accumulation of CO_2 in the blood during sleep. This relative acidosis tends to disappear during the course of the morning, due to the reactivation of respiratory center with a fall of CO_2 in the blood. Watson[4] has suggested that the same process may be a factor in causing an increase in the blood and urine sugar during the early part of the day.

Free hydrochloric acid is constantly absent in pernicious anemia. The achlorhydria is generally regarded as a diagnostic criterium of pernicious anemia. It is of interest in this connection to note that the fasting blood sugar in 16 patients with pernicious anemia was found by Johnson[5] to be above normal. Rennie[6] performed glucose tolerance tests in 19 patients with pernicious anemia and found definitely abnormal tolerance curves in 58 per cent (11 cases), 8 of these having prolonged curves, 3 abnormally high. The abnormality of the curves had no relation to the hemoglobin, red blood cell count, age, weight, temperature, or pulse rate. It would not be irrational to account for these curves by the abnormal physiologic activity of the digestive apparatus.

Watson[7] has obtained in 6 patients with achlorhydria sugar tolerance curves that speak for a disturbed carbohydrate metabolism. In 3 of them an intermittent glucosuria was the only symptom suggestive of a defective carbohydrate tolerance.

Dr. D. Meranze and I carried on some observations on nondiabetic hospital patients from Dr. H. B. Shmookler's service of the Mount Sinai Hospital, that would tend to indicate the influence of the administration of hydrochloric acid on sugar tolerance curves. Patients were selected who have shown an absence of free hydrochloric acid and who have shown no disturbance in carbohydrate metabolism. One and seventy-five hundredths gm. of glucose per each kilogram of body weight was given by mouth on empty stomach. The glucose was dissolved in 400 c.c. of water and flavored with lemon juice. Samples of blood for blood-sugar estimation were withdrawn in the fasting state and then every half hour for the first hour and two hours later. Blood-sugar determinations were made by the Folin-Wu Micro blood-sugar method. Two days later the same amount of glucose was given and in

addition the patient drank 5 c.c. of dilute hydrochloric acid with the glucose; and one hour later another dose of 5 c.c. of dilute hydrochloric acid was given. Table II illustrates the results of some of these experiments.

TABLE II

SUGAR TOLERANCE TEST

Fasting Blood	93	mg. per 100 c.c. of blood
½ hr. after ingestion of glucose	128	mg. per 100 c.c. of blood
1 hr. after ingestion of glucose	171.5	mg. per 100 c.c. of blood
2 hr. after ingestion of glucose	99	mg. per 100 c.c. of blood

CASE 4.—Mr. M. T. Free HCl 0; total acidity max. 15.
Glucose plus Hydrochloric Acid (5 c.c.).
Another 5 c.c. was given at end of first hour.

Fasting Blood	120	mg. per 100 c.c. of blood
½ hr.	248	mg. per 100 c.c. of blood
1 hr.	278	mg. per 100 c.c. of blood
2 hr.	171	mg. per 100 c.c. of blood

SUGAR TOLERANCE TEST

Fasting Blood	80	mg. per 100 c.c. of blood
½ hr. after ingestion of glucose	111	mg. per 100 c.c. of blood
1 hr. after ingestion of glucose	129	mg. per 100 c.c. of blood
2 hr. after ingestion of glucose	120	mg. per 100 c.c. of blood

CASE 7.—Mr. J. W. Free HCl 0; total acidity max. 20.
Glucose plus Hydrochloric Acid (5 c.c.).
Another 5 c.c. was given at end of first hour.

Fasting Blood	110	mg. per 100 c.c. of blood
½ hr.	218	mg. per 100 c.c. of blood
1 hr.	206	mg. per 100 c.c. of blood
2 hr.	185	mg. per 100 c.c. of blood

Comment: Achlorhydria has been found in from 4 to 6 per cent of apparently normal persons.[8] This agrees with the work of Bennett and Ryle,[9] who in 100 medical students of an average age, twenty years, demonstrated a complete absence of free hydrochloric acid in four persons. Other investigators: Lockwood (quoted by Alvarez[8]), Eggleston[10] encountered achlorhydria in from 6 to 10 per cent of normal cases.

The occurrence of achlorhydria and hypoacidity in diabetes mellitus is of greater frequency than normally found. In our study, achlorhydria occurred in about 33.3 per cent of the cases and hypoacidity in 21.2 per cent. That occurrence of achlorhydria in diabetes mellitus has not been given its proper valuation may be gleaned from Faber's[11] work. He states that of all achlorhydria individuals 10 per cent develop pernicious anemia and 90 per cent may suffer from exophthalmic goiter, arthritis deformans, cholecystitis. He fails to mention diabetes mellitus.

The relative frequency of achlorhydria and hypoacidity is indicated in Table III.

Bowen and Aaron,[12] and Joslin,[13] found achlorhydria in 27.3 per cent of cases. The incidence of achlorhydria in diabetes including this group is 28.7 per cent. A prominent symptom in Bowen and Aaron's patients was the diarrhea. According to them, diarrhea was not noted unless achlorhydria

TABLE III

GASTRIC ACIDITY IN DIABETES ACCORDING TO THE AGE OF PATIENT AND DURATION OF DISEASE

NUMBER OF PATIENTS	DURATION OF DISEASE	AVERAGE AGE	SEX		FREE HYDROCHLORIC ACID		
			MALE	FEMALE	NORMAL OR ABOVE	DIMINISHED	ABSENT
			Bowen and Aaron				
46	Under 5 years	49	16	30	29	8	9
16	From 5 to 10 yr.	53	3	13	4	6	6
4	From 10 to 15 yr.	50	2	2	1	0	3
2	From 15 to 20 yr.	66	1	1	1	0	1
1	From 20 to 25 yr.	64	0	1	0	0	1
			Joslin				
10	Under 5 years	46	4	6	8	0	2
10	From 5 to 10 yr.	53	3	7	6	1	3
7	From 10 to 15 yr.	55	1	6	5	0	2
5	From 15 to 20 yr.	58	1	4	4	0	1
5	From 20 to 25 yr.	55	3	2	4	0	1
			Wohl				
11	Under 5 years	48	4	7	7	2	2
14	From 5 to 10 yr.	51	5	9	6	3	5
8	From 10 to 15 yr.	54	3	5	2	2	4
139			46	93	77, or 55.4%	22, or 16%	40, or 28.7%

existed. According to our study diarrhea was not a frequent symptom. Of the 11 patients with achlorhydria, one patient complained of diarrhea, and in another was an alternating diarrhea and constipation. In the patient with diarrhea, this was checked by the administration of dilute hydrochloric acid. In the second a colitis was demonstrated by sigmoidoscopic examination. It is interesting to note that the patients showing achlorhydria had diabetes of a long duration. The known causes for achlorhydria and hypoacidity such as cholecystitis, tuberculosis, carcinoma of the stomach, and pernicious anemia, have not played a rôle in our patients, as in only two patients with achlorhydria, there was demonstrated a chronic cholecystitis.

The relationship between the chloride metabolism and the secretion of free hydrochloric acid in the stomach is well established. It is significant that in diabetes one frequently finds a diminution of chlorides in the pancreatic secretions. Myer-Bish[14] has noted in diabetes a close parallelism between the reduction of pancreatic ferments and the chlorides in the pancreatic juice.

Lee Foshay[15] has also observed a reduction of the serum chloride concentration with a corresponding increase of the chloride in the blood corpuscles when the blood sugar was increased, in other words, in diabetes the chloride is in a less available form for formation of hydrochloric acid by gastric glands than in health.

CONCLUSIONS

In the treatment of patients with diabetes mellitus attention should be given to the gastrointestinal tract. In a respectable number of patients an achlorhydria and hypoacidity may be demonstrated.

The administration of dilute hydrochloric acid in addition to the diabetic regimen may lessen the gastric complaints in such persons.

There is some clinical and laboratory evidence of the relationship of a disturbed gastric function to disturbed carbohydrate metabolism. It suggests itself that in the interpretation of abnormal sugar tolerance curves one should also consider the physiologic function of the digestive tract.

I wish to express my appreciation to Dr. Wm. Egbert Robertson for his many valuable suggestions in the preparation of the work and to Dr. Herman Jahr of Omaha, Nebraska, and Dr. D. Meranze for technical assistance.

REFERENCES

1. Watson, E. M.: Factors in the Interpretation of Anomalous Blood Sugar Curves, Canad. M. A. J. **17**: 1036, 1927.
2. Cammidge, P. J., and Howard, H. A.: New Views on Diabetes Mellitus, London, 1923, Henry Frewde and Hadderand Stoughton, p. 27, 34, 42.
3. Leathes, J. B.: Brit. M. J. **2**: 165, 1919 and Lancet **2**: 933, 1920.
4. Watson, E. M.: Concerning Certain Factors Which May Influence the Sugar Content of the Blood and Urine, J. LAB. & CLIN. MED. **15**: 234, 1929.
5. Johnson: Acta med. scandinav. supp. 3, 1922.
6. Rennie, Thomas A. C.: Glucose Tolerance in Pernicious Anemia, J. LAB. & CLIN. MED. **16**: 557, 1931.
7. Watson, E. M.: loc. cit.
8. Alvarez, Walter: The Oxford Medicine **3**: Diseases of the Stomach.
9. Bennett, T. I., and Ryle, J. A.: Studies in Gastric Secretion, Guy's Hospital Ref. **71**: 286, 1921.
10. Eggleston, E. L.: Gastric Secretory Disturbance, 20-89, May, 1925. Bull. Battle Creek Sanitarium and Hospital.
11. Faber, K.: Berl. klin. Wchnschr. **1**: 958, 1913.
12. Bowen, B. D., and Aaron, A. H.: Gastric Secretion in Diabetes Mellitus, Arch. Int. Med. **37**: 675, 1926.
13. Joslin, E. P.: Nelson Loose Leaf Medicine, 1929, p. 109 K, New York, Thomas Nelson & Sons.
14. Myer-Bisch, R.: (discussion) Verhandlungen der Gesellschaft fur Verdauungs und Stoffwechselkrankheiten. IX, Tagung, in Berlin (16. bis. 18. Oktober, 1929), 1930, Georg Thieme, Verlag, Leipzig.
15. Foshay, Lee: Relation of Hyperglycemia to Relative Blood Volume, Chlorine Concentration and Chlorine Distribution in the Blood of Dogs, J. Exper. Med. **42**: 89, 1925.

NOTE: Since the preparation of the manuscript, an article appeared by Howard F. Root, Diabetes and Pernicious Anemia, J. A. M. A. **96**: March 21, 1931, dealing with the incidence of Achlorhydria in Diabetes Mellitus.

SEPTIC CAVERNOUS SINUS THROMBOSIS*

Report of Two Cases With Recovery of One Following Bacteriophage Therapy

By B. F. Stout, M.D., San Antonio, Texas

ACUTE infectious cavernous sinus thrombosis is a relatively rare disease if one considers the number of cases reported in the literature. However, it is possibly more common than these reports show, cases occurring which are either not reported or not recognized as such.

The most extensive treatise on this subject is the book by Eagleton,[1] who in 1926 collected the reports up to that time and added in detail 25 cases of his own. Smith,[2] in 1918, reviewed the literature and found less than 300 cases reported up to that time. Dixon,[3] in 1926, reported 10 cases, one with recovery, but which he regarded as an error in diagnosis.

Regarding the prognosis, Dwight and Germain,[4] in 1902, collected 182 cases from the literature and found that 7 per cent of these patients recovered spontaneously. Babbit,[5] commenting on this, stated "that 7 per cent recoveries must raise the question of diagnosis without autopsy." Smith[2] expressed the opinion that "on the assumption that the clot in the sinus was not infected and considering the great number of observers, the fragmentary character of many of the reports, and the possibilities of inaccuracies and of errors in diagnosis, we are forced to the conclusion that thrombosis of the cavernous sinus is practically always fatal if the thrombus is infected and not drained." Dixon concluded "that meningitis follows so closely after the eye symptoms develop that septic thrombosis of the cavernous sinus is a non-surgical complication and that reported cases of recovery were probably errors in diagnosis."

This paper has to do with the report of two cases, the first with classical symptoms, which recovered completely without sequelae and in which radical surgery was not employed, but dependence placed on the use of antistaphylococcus bacteriophage filtrates and blood transfusions. A second case, also typical, but which came under observation too late for any hope of recovery.

Case 1.—Patient, L. D., female, aged sixteen. Family history negative. Past history: Perfect health up to the time of the present illness. On July 19, 1930, a small furuncle appeared on the center of the chin just below the border of the lip and was treated by local application of hot fomentations. On July 23 it was lanced, little pus being found. The lip began rapidly swelling and she entered the Medical Arts Hospital on the next morning with a temperature of 101.2° which rose to 103.2° the same day. One c.c. of bacteriophage was injected under the skin and a wet dressing of it used locally. On the following day the lesion was cauterized deeply with the thermocautery and the bacteriophage

*Received for publication, April 3, 1931.

treatment repeated. On the following day, July 26 (the seventh day), the swelling had rapidly spread up the right side of the face, with continued high temperature to 105.6°. Because of the cellulitis with no pus forming in the lesion, 50 c.c. of antistreptococcus serum was given in the belief that it was a streptococcus infection.

On July 28 (the ninth day) the first blood count was made showing a low total leucocyte count of 7,800 but with 96 per cent neutrophiles, 57 per cent of which were immature forms constituting a dangerous left nuclear shift. A blood culture and cultures from the lesions were made. All showed Staphylococcus aureus, the blood in one c.c. amounts in 30 c.c. tubes of broth yielding a heavy growth in ten hours. By the next morning (the tenth day) swelling was intense over the right side of the face, involving the right eye which showed exophthalmos and chemosis. The right side of the neck was also brawny and hard. The temperature rose to 103.4° and the blood continued to show a most dangerous picture.

By July 31 (the thirteenth day) the left eye was involved but to a less extent than the right, which showed now extreme exophthalmos and chemosis. The thrombus had invaded the left side of the sinus. The lids were swollen shut, were a bluish-purple, pitted on pressure and could not be opened enough to permit an examination of the eyegrounds. The tension was so great that pressure necrosis was feared, and it was decided to make superficial linear incisions in the lids and conjunctiva to relieve the tension. This was done together with a small incision in the swollen cheek. No pus was found. Reference to Table I will show the extreme gravity of the blood picture from day to day, the temperature range, and the therapy used.

Bacteriophage was at first used subcutaneously and locally, but in view of the heavy blood stream infection, in addition to the rapid increase in symptoms, it was decided to use it intravenously. This was done daily with doses morning and afternoon on three of the days. July 29 (the tenth day) 4 c.c. were given subcutaneously and on the thirtieth, 9 c.c. in divided doses were given under the skin. The intravenous treatment was well borne, being at first followed by short but definite chills, those later becoming mild. On August 3 (the sixteenth day) urticaria with painful joints developed and because the intravenous injections of bacteriophage greatly aggravated these symptoms, it was abandoned. The urticaria was undoubtedly caused by the antistreptococcus serum which was given on July 26. A blood culture taken at this time and cultured both in broth and on agar plates remained sterile after seven days incubation.

Reference to Table I will show the number of blood transfusions and the use of parathormone and calcium given for the control of the urticaria and other therapy. The total leucocyte count began to rise though the neutrophiles and nuclear shift did not show much improvement. An occasional eosinophile appeared which may have been due to urticaria.

By August 6 (the nineteenth day) the swelling in the neck and face had begun to improve and the wound in the lower lid showed signs of healing as did the superficial incisions in the eyelids. The exophthalmos and chemosis in both eyes was still marked but the lids could now be separated for examination of the eyegrounds. The pupil of the right eye was somewhat dilated but the ophthalmologic examination showed nothing but congestion of the retinal veins while the nerve and macula appeared normal in both eyes. The patient was still unable to voluntarily raise the lids of either eye. From this time until August 22 the swelling except for bulging of the eyes had subsided completely, with healing of the incisions. The patient's mind was clear and her appetite good. However, the blood picture showed higher total counts of the leucocytes, a marked left nuclear shift, and she had occasional delirium when asleep.

The temperature was somewhat lower in range but headache with pain in the back of the head grew increasingly severe until on August 23 (the thirty-second day) she became very ill with vomiting and severe pain in the back of the head and neck. The leucocytes rose to 20,900 and the temperature to 103.2°. At 4:00 P.M. a lumbar puncture was made yielding 15 c.c. of a turbid fluid under high pressure. The cell count showed 1100 per c.mm., while direct smears and cultures showed numerous staphylococci. One c.c. of bacteriophage was introduced into the spinal canal. Since I had no precedent for the intraspinous use of bacteriophage I decided on 1 c.c. as a trial dose. A blood culture taken at this time

TABLE I

CASE NO. 1

DATE	HEMOGLOBIN PER CENT	RED CELLS IN MILLIONS	TOTAL WHITE CELLS	HEMOGRAM NORMALS									TEMPERATURE RANGE 24 HR.	BACTERIOPHAGE THERAPY	OTHER MEDICATION	REMARKS
				% = 0 MYL.	% = 0 JUV.	% = 2-5 STABS	% = 60-70 SEG.	TOTAL NEUTROPHILES	% = 2-5 EOS.	% = 1 BOS.	% = 20-30 LYMPH.	% = 6-8 MON.				
7/24/30													101.8 to 103.2	1 c.c. subcutaneous local application.	Fomentations.	Entered hospital lip much swollen.
7/25/30													100 to 104.6	1 c.c. hypo. local dressing.	Thermo cautery.	Increasing severity of swelling.
7/26/30													101.2 to 105.6	50 c.c. anti-streptococcus serum.	Fomentations.	Swelling spreading up side of face.
7/27/30													102 to 104	1 c.c. hypo. local dressing.		Right eye beginning to be involved.
7/28/30			7,800	5	12	40	39	96	0	0	3	1	101.2 to 105	1 c.c. hypo. local dressing.	300 c.c. glucose (25 per cent) intravenously.	Culture made from blood and lesions. Increasing swelling of right eye.
7/29/30	80	4.18	8,900	3	13	36	38	91	0	1	6	3	99 to 103.4	1.5 c.c. intravenous. 4 c.c. hypo.	Blood transfusion 250 c.c	Blood cultures show growth in 10 hr. Exophthalmos-chemosis swelling of face and neck. Chill followed phage injection.
7/30/30			6,700	15	17	43	20	95	0	0	1	4	100 to 103.4	1.5 c.c. intravenous. 9 c.c. hypo. in divided doses.	Blood transfusion 250 c.c.	Light chill followed intravenous phage.
7/31/30			9,700	10	15	36	33	94	0	0	5	1	101.8 to 104.2	1.5 c.c. A.M. and P.M. 5 c.c. hypo.		Left eye involved, incisions made in lids and cheek.

TABLE I—CONT'D

8/ 1/30	80	4.27	8,940	3	14	42	26	85	0	0	8.5	6.5	102 to 105.6	1.5 c.c. intravenous. 4 c.c. hypo.	Blood transfusion 250 c.c.	Urticaria severe.
8/ 2/30			10,300	4	12	41	19	76	1	1	16	6	101.2 to 104.4	1.5 c.c. A.M., 2 c.c. P.M.	Parathormone and calcium gluconate.	Urticaria, adrenalin given for relief.
8/ 3/30	80	3.88	13,000	2	13	56	14	85	1	0	10	4	99 to 103.6	1.5 c.c. intravenous.	Parathormone and calcium and adrenalin	Blood culture sterile, phage treatment suspended because of urticaria.
8/ 4/30			14,500	0	10.5	41	30	81.5	1.5	0	11	6	101 to 103.6			Pain in head, urticaria, slight delirium.
8/ 5/30	75	3.75	10,500	3	3	40	36	82	1	1	12	4	101.8 to 103.8		5 c.c. metaphen intravenously.	
8/ 6/30	70	3.45	11,250	0	8	40	33	81	1	0	9	9	101.2 to 104.6	1 c.c. hypo.	Blood transfusion 250 c.c.	Phage caused slight return of urticaria, some delirium.
8/ 8/30	80	4.82	14,200	1	4	32	53	90	0	0	4	6	101.2 to 104		8 c.c. metaphen intravenously.	Swelling of neck and face improved. No improvement of eyes. Pain in head, mild delirium.
8/ 9/30			14,740	0	3	35.5	47.5	81	0	0	13	6	99.6 to 103		Parathormone and calcium, metaphen 5 c.c.	
8/10/30	75	3.97	13,450	2	3	33	47	85	0	1	12	2	101.4 to 103.6		Blood transfusion 250 c.c	Much headache.
8/11/30	78	3.99	18,000	0.5	13	29	49	91.5	1	0	5.5	2	99 to 103		200 c.c. buffered saline and glucose intravenously.	Much pain in hip and shoulder. Slight delirium.
8/12/30	72	4.30	18,890	0.5	8	38	41	87.5	0	0	8.5	4	100 to 104.4		Blood transfusion 250 c.c.	Pain in hip.
8/13/30	72	4.57	16,800	1.5	2.5	46.5	41.5	87	1	0	7	5	101 to 105		200 c.c. buffered saline and glucose.	Pain in head and right ear.
8/14/30	80	4.14	13,000	2	3	39	46.5	90.5	0.5	0.5	5.5	3	100 to 103.6			

TABLE I—CONT'D

DATE	HEMOGLOBIN PER CENT	RED CELLS IN MILLIONS	TOTAL WHITE CELLS	HEMOGRAM NORMALS									TEMPERATURE RANGE 24 HR.	BACTERIOPHAGE THERAPY	OTHER MEDICATION	REMARKS
				% = 0 MYL.	% = 0 JUV.	% = 2-5 STABS	% = 60-70 SEG.	TOTAL NEUTROPHILES	% = 2-5 EOS.	% = 1 BOS.	% = 20-30 LYMPH.	% = 6-8 MON.				
8/15/30	80	4.14	15,600	0.5	2.5	28	52.5	83.5	5	0	11.5	4.5	99 to 103.8			Pain in back of neck and right ear.
8/16/30	70	4.30	13,750	0.5	2.5	29.5	49	81.5	1	0	10.5	7	99 to 101			Pain in back of head and neck. General body soreness.
8/18/30	75	4.55	13,500	0.5	0	20	58	78.5	0	0	11.5	10	99 to 100			Swelling in face almost gone, lip normal and eye incisions healed. Pain in head.
8/22/30	72	3.75	15,500	0	1	17	65.5	83.5	0.5	1	13	3	101 to 102.4		Codeine and aspirin.	Increasing pain in head and neck. General body soreness.
8/23/30	73	3.74	20,900	1	3	27	53	78	0	0	11	11	101.2 to 103.2	1 c.c. injected into spinal canal.	Codeine and aspirin.	Severe pain in back of head and neck, nausea and vomiting. Lumbar puncture. Turbid fluid cell count 1100. Staph. found smears and cultures.
8/24/30	77	4.30	13,400	0	0	9	76	85	0	0	6	9	99.8 to 101.2	4 c.c. intraspinal.	Codeine and aspirin.	Pain much less, nausea less. Spinal fluid shows 370 cells. Blood culture sterile.
8/25/30	76	4.36	11,400	1	1	20	66	88	2	0	9	1	98 to 99.6	5 c.c. intraspinal.	Codeine.	Cell count of fluid was 320, culture showed one colony on plate. Less headache, sleeping much.
8/26/30	70	3.76	12,000	1	1	12	72	86	1	0	7	6	97.4 to 99	5 c.c. intraspinal.	Codeine.	Cell count of fluid was 276. Mind clear, sleepy but still pain in head.

TABLE I—CONT'D

8/27/30	74	4.11	15,240	1	1	13	72.5	87.5	0	0	6.5	6	97.4 to 99	5 c.c. intraspinal.	Codeine.	Fluid bloody from trauma. Culture fluid sterile sleeping much, appetite better.
8/28/30	73	3.78	12,400	1	2	16	66	85	1	0	10	4	99 to 101.2	5 c.c. intraspinal.	Codeine.	Fluid bloody from trauma. Pain in head. Culture fluid sterile.
8/30/30	70	3.48	8,500	0	0	12	61	73	1	0	21	5	98.6 to 101	1 c.c. hypo. no reaction.	Codeine.	Still some pain in head.
9/ 1/30	70	3.80	8,150	0	0	8	62	70	0	0	18	12	98.2 to 101.2	1 c.c. hypo.	Codeine.	Urine shows pus 2+.
9/ 3/30	73	3.90	11,000	0	1	19	54	74	0	0	20	6	98.2 to 101.4	5 c.c. intraspinal.	Codeine.	Urine shows 4+ pus with staph. and B. coli. Pains in head, nausea.
9/ 4/30			12,300	0	0	15	65	80	0	0	16	4	99.4 to 101.6	Mixed staph. and coli phage into ureters.	Cystoscopy.	Urine each kidney shows staph. and B. coli. Much pus.
9/ 5/30			6,440	0	0	5	65	70	1	1	24	4	99.8 to 101	1 c.c. hypo.	Codeine.	Bladder irrigation, urine shows 2+ pus.
9/ 6/30	70	3.70	12,000	0	0	6	66	72	0	0	24	4	99.4 to 101.6	1 c.c. hypo.	Codeine.	
9/12/30	70	3.46	10,200	0	0	10	56	66	1	0	30	3	97.4 to 102	1 c.c. hypo.	Codeine.	Body soreness and pain in head and hip.
9/13/30			16,500	0	2	31	41	74	1	0	12	13	99 to 102.6	1 c.c. hypo.	Codeine.	
9/15/30			17,350	0	0	32	55	87	0	0	9	4	99 to 104.2	1 c.c. hypo.	Codeine.	Much nausea.
9/16/30			14,500	2	10	17	63	92	0	0	4	4	100.8 to 105	2 c.c. intraspinal.	Codeine.	Nausea, pain in back of head. Blood culture sterile.
9/17/30			25,100	0	8	19	46	73	1	1	11	14	101.4 to 104	1 c.c. hypo. up to Oct. 1.	Codeine.	Continued soreness and headache.
10/ 1/30	70	3.63	22,800	0	0	16	57	73	0	0	21	6	99.8 to 103.4		Codeine.	Nausea, urine 4+ pus and B. coli.
10/ 2/30 to 10/ 8/30													97.6 to 98.6		None	Small amounts of pus in urine. Rapid general improvement.

In this chart the Schilling hemogram is used: Myelocytes (myl.); juveniles (juv.); stabs or rod-nuclears and segmented or mature neutrophiles (seg.). The sum of these is entered as total neutrophiles. The normal percentage is shown and the increase of the immature forms indicates the "shift to the left."

gave no subsequent growth. The next morning at 9:00 A.M. the blood count showed 13,400 leucocytes. The shift was more to the right and the patient felt considerably better. At 11:00 A.M. a lumbar puncture was made, the fluid being less under pressure while the cell count showed now 370 cells. Since no reaction followed the 1 c.c. dose, 4 c.c. of bacteriophage were injected into the spinal canal. The temperature rose only to 101.4° following this. Lumbar punctures were made daily for the following four days, each time 5 c.c. of bacteriophage being introduced after the fluid was withdrawn. Only 10 c.c. of fluid was withdrawn because of the fear of disturbing the lesions above.

The patient improved rapidly, sleeping much of the time, the appetite returned, but she complained continually of headache, which was controlled by codein. The temperature dropped close to normal after the second intraspinous injection of bacteriophage and the blood picture improved. On September 3 vomiting and pain in the head and right eye returned; however, the temperature range was not high, and the blood picture was nearly normal. Another lumbar puncture was made, showing a clear fluid under normal pressure and with a cell count of 16. Cultures from the fluid yielded no growth after seven days' incubation. Again 5 c.c. of bacteriophage were given intraspinously. The temperature rose to 101.4°. The urine, which had been examined daily since the patient's entrance to the hospital, at this time showed a large amount of pus with staphylococci and B. coli. On September 4 a cystoscopy and ureteral catheterization was done, the urine from each kidney showing pus with staphylococci and B. coli. Mixed staphylococcus and coli bacteriophage was introduced into the kidney pelvis. Following this the urine showed variable amounts of pus from 2- to 4-plus. Local bladder irrigations and instillations were made daily.

Bacteriophage treatment by subcutaneous injection was resumed on September 13 (the fifty-third day) and was followed by no reaction of any kind. The blood picture from day to day showed an increasing leucosis, a variable neutrophilia and a marked left nuclear shift. The patient had, of course, rapidly lost weight from the first and by now was emaciated and extremely weak. The eyes, following the intraspinous injections of bacteriophage had rapidly improved with ability to voluntarily raise the lids, the exophthalmos subsided, vision was good with the exception of diplopia, and the patient was in good spirits. The temperature reached nearly normal in the mornings but rose each afternoon. On September 15 (the fifty-fifth day) the temperature rose to 103.8°, the leucocytes to 17,350 and nausea and vomiting with headache returned. A lumbar puncture yielded a clear fluid under normal pressure with a count of 21 cells. Cultures of the fluid were again sterile. Another blood culture was made which gave no growth. Two c.c. of bacteriophage were injected into the spinal canal.

The urine at this time showed a 4-plus pus with B. coli but the fear that the infected sinus was still causing the symptoms, in spite of negative spinal fluid findings, was so strong that treatment of the kidney pelves was not done until October 1 (the seventy-first day). On this day nausea reappeared with chilly sensations, the total leucocytes rose to 22,800 though the neutrophiles were only 73 per cent and the nuclear shift not marked.

The ureters were catheterized, pus being found from the right ureter, and argyrol was injected into the kidney pelvis. Following this the temperature dropped to normal and remained so until the patient was discharged from the hospital on October 8 (the seventy-eighth day). No further blood counts were made, the eyes were nearly normal and the pus content of the urine dropped to a few cells. After the patient returned home she had a few slight rises in temperature with some congestion about the eyes, these attacks being coincident with her menstrual periods, and by the first of December she weighed ten pounds more than before her illness and had normal vision and no remaining effects of the infection.

CASE 2.—Patient, Mrs. K. C., aged thirty-four, entered the Robert B. Green Memorial Hospital September 18, 1930, at 2:00 P.M. Service of Dr. Dudley Jackson.

Past History: During past three months she had recurring attacks of furuncles about the face. September 11 she had a small one on the left lower lip. On the fourteenth (third day) she picked it, and the next day the face and lip began swelling, increasing in severity until she entered the hospital where examination showed the whole left side of the face to be swollen, including the left eye which she was unable to open. The lids were

edematous and bluish-red in color. There was chemosis and a small amount of exophthalmos present. She complained of severe pain in the head and showed much mental confusion. The temperature rose from 100.8°, on entrance, to 104.4° in the night. On the following day (the fourth day) the patient became delirious with increase in the swelling of the face and with chemosis present in both eyes.

A lumbar puncture showed a slightly turbid fluid with 72 cells and a few staphylococci on direct smear. The leucocytes were 16,400 with 77 per cent of neutrophiles (the Schilling count was not made). At 2:00 P.M. 2 c.c. of antistaphylococcus bacteriophage were given subcutaneously. The patient continued to grow rapidly worse during the night, and the following morning, September 20 (the ninth day) 2 c.c. of bacteriophage were given subcutaneously. Following a lumbar puncture at 2:30 P.M. which showed a purulent fluid, 1 c.c. of phage was given intraspinously. The temperature rose to 104°, the pulse to 140 and at 4:00 P.M. she died, having been in the hospital about forty-eight hours.

At autopsy (Dr. Dudley Jackson), there was found on opening the skull, a general meningeal exudate with a collection of purulent material in the region of the left frontal lobe of the brain. Both orbital cavities were filled with an infected edematous material with thrombosis of the orbital veins. The longitudinal sinus contained an infected thrombus and on opening the cavernous sinuses both were found filled with a purulent soft clot. The pleural cavities on both sides contained a seropurulent exudate, while the lungs were filled with infected emboli of various sizes from small areas to larger consolidations. The pus from the cavernous sinuses and other lesions yielded pure cultures of Staphylococcus aureus. The other organs of the body showed nothing of importance.

DISCUSSION OF CASE I

This case report presents several outstanding points of interest and importance.

First, that a patient with a classical picture of infectious cavernous sinus thrombosis with septicemia and meningitis should survive at all.

Second, the sterilization of a heavy blood stream infection by intravenous bacteriophage therapy.

Third, the control of a Staphylococcus meningitis by intraspinal use of bacteriophage.

Fourth, the faithfulness with which the Schilling index of the leucocytes mirrored the clinical facts in the case.

The chief disease simulating cavernous thrombosis is orbital cellulitis. Eagleton[1] and Babbitt[5] have discussed this question and pointed out the differential points in diagnosis. In orbital cellulitis the disease nearly always starts in the nasal sinuses or from direct trauma to the eye. The serious blood picture in this case, persisting in spite of the subsidence of external signs of infection with the exception of the eyes, indicated with certainty the presence of a focus somewhere in the brain or venous sinuses. The appearance of delirium during the days preceding the development of meningitis, as shown by the spinal fluid and the severe headache, according to Eagleton[1] do not appear when the infection is confined to the sinus. He states that "the appearance of delirium is of the gravest significance." The lungs and endocardium at no time showed any involvement and no other metastatic foci developed during the illness.

Concerning the question of spontaneous recovery, reference to what has been said about prognosis in this paper, together with the history of an infected sinus thrombosis, septicemia and meningitis, casts grave doubt as to

its possibility in this case. In the treatment of this patient, aside from six blood transfusions and other supportive measures, the one hope was centered on the specific treatment by antistaphylococcus bacteriophage. Faith that this might be of aid in spite of the desperate nature of the case was inspired by previous experience with less alarming yet severe staphylococcus infections.[6]

In this case the infection progressed so rapidly and with such alarming symptoms, including a blood picture that showed an overwhelming toxic effect on the bone marrow, that the first attempts to cope with the infection by subcutaneous and local use were soon abandoned for intravenous injections. These were at first followed by definite chills but later injections were well borne until the severe urticaria and joint pains forced the suspension of bacteriophage therapy for the time. The urticaria was undoubtedly due to the previous use of the antistreptococcus serum and was aggravated by the bacteriophage injections. However, following six days of intravenous bacteriophage therapy a blood culture showed the blood to be sterile.

Lowenstein[7] has quoted Stetson as saying that the staphylococcus is the deadliest organism encountered in general sepsis; also that Peet, Reed and Stiles and others have stressed the almost invariably fatal outcome of Staphylococcus septicemia when secondary to furuncles. He reported one case of encephalitis in which Staphylococcus albus was isolated from both the blood and spinal fluid. The patient recovered following intramuscular, intravenous and intraspinal injections of staphylococcus antitoxin. However, in two other patients treated by this method, the first died on the day after entering the hospital; while in the other, 7 intravenous injections failed to have the slightest effect in preventing the fatal termination. He called attention to the fact that the staphylococcus is very irregular in the production of soluble toxin upon which dependence must be placed for the production of antitoxin. In two of his cases he used nonspecific protein without success and commented on the little encouragement given in the literature to this form of therapy.

Rice,[8] in reporting two cases of Staphylococcus septicemia, stated that both died, though one lived weeks longer than was expected. Local and subcutaneous treatment by bacteriophage was employed, fear of the peptone in the filtrate preventing the intravenous use of it.

The early sterilization of the blood stream in this case, I believe, prevented the development of metastatic foci in the lungs which are so frequently found, as well as elsewhere. It also localized the infection and confined it to the original sites. This action of bacteriophage in less serious infections has been noted by numerous observers as a very common and characteristic result. I have seen numerous cases of severe cellulitis of the face arising from foci about the mouth or nose in which local and subcutaneous use of the bacteriophage caused rapid subsidence of the cellulitis with localization, relief of pain, and prompt termination of the infection. The infection in this case was obviously confined to the sinuses, with a local meningitis, until later when it became generalized. The most spectacular evidence of the rôle of bacteriophage in the recovery of this patient was shown by the rapid and complete control of the meningitis by the intraspinal method of administration.

In both the intravenous and intraspinal methods direct contact was assured with the organisms causing the infection, constituting the ideal method of approach. While the peptone in the filtrate may be an obstacle to the use of large amounts intravenously, the harmless nature of this agent used locally is such that the entire meningeal cavity could probably be filled with it, replacing the spinal fluid.

The therapeutic value of antistaphylococcus bacteriophage filtrates has been much in dispute. However, such favorable reports have been published by d'Herelle,[9] Bruynoghe and Maison,[10] Gratia,[11] Gougerat and Payre,[12] Hauduroy,[13] Grenet and Isaac-Geroges,[14] Bazy,[15] Lingeman,[16] Raiga,[17] Rice,[8] Larkum,[18] Alderson,[19] Crutchfield and Stout[6] and others, that the mass of evidence supporting the therapeutic value of this agent cannot be ignored.

D'Herelle[9] states that "too much emphasis cannot be placed on the fact that bacteriophage acts effectively only when very virulent races are used." Nelson[20] has studied the effect on phagocytosis by injecting rabbits intravenously with 5 c.c. of bacteriophage together with a strain of staphylococcus susceptible to lysis and found an immediate and marked increase in the phagocytic index. With a strain of staphylococcus not lysable the phagocytic index was not altered. Smith[21] has shown in a similar study that the degree of phagocytosis is determined not only by the period of contact with bacteriophage but also by the virulence of the lytic principle employed and that the bacteria become more susceptible to phagocytosis after contact has been interrupted. D'Herelle,[9] Gohs and Jacobsohn[22] and others, have also demonstrated the marked effect on phagocytosis. Arnold and Weiss[23] found that a single dose of bacteriophage lysed bacterial filtrate developed a rapid increase in the antibody titer of the rabbit and was able to protect the animal from a lethal dose of the homologous organism. The bacteriophage lysed organisms being split products were thus shown to have a more rapid and active antibody forming power than vaccines or autolysates. Vaccines have been regarded as helpful but not curative in Staphylococcus septicemia and then only in chronic cases.

The race of bacteriophage used in this case was one of high virulence, having a titer of 10^{-8}, and has been capable of lysing more than 90 per cent of the strains tested. The strain recovered from the lesions of the patient was readily susceptible to its action. It is my belief that this patient survived only because of early and vigorous treatment, particularly by the intravenous method. In giving this patient only 1 c.c. doses subcutaneously during the first five days, much time was lost. Much larger amounts should have been used, given twice or three times daily, and earlier intravenous treatment instituted.

If confronted with a case such as Case 2, in which meningitis has become generalized and septic emboli have already invaded the lungs, no hope can be entertained of recovery by any form of treatment. This patient had been subject to staphylococcus infection which lowered her resistance and rendered her more vulnerable to the final invasion of vital structures. Had she been fortunate enough to have been seen early and vigorous and fearless bacteriophage treatment been instituted, she probably would have survived.

Reference to the history of Case 1 will show how narrow a margin of chance the patient had during the first days of the infection. The repeated injections subcutaneously of large doses together with the intravenous dosage produced such a resistance that the breaking through of the infection into the meninges did not take place until more than three weeks after the bacteriophage therapy began. It would seem clear that the study of the behavior of this patient under bacteriophage treatment should teach more than that one case of its type has recovered following its use. These cases are uncommon, but it is well known that staphylococcus infections about the face, lips, or nose are common, and all potentially dangerous. These can be promptly and certainly relieved by early and adequate bacteriophage treatment.

The success in sterilizing the blood stream in this case should open a field of usefulness in Staphylococcus septicemia from other foci. It should, of course, be combined with such surgery as seems indicated in each case and should be used locally, subcutaneously and intravenously. Doses up to 5 c.c. at a single injection can be used subcutaneously, while from 1 to 2 c.c. intravenously were safely employed in this case. Nelson[20] used daily intravenous doses in rabbits of 5 c.c. without apparent harm.

It is hoped that the success attending the use of bacteriophage in Case 1 will stimulate further study and experiment in this class of infections and lead to the saving of some at least of these otherwise hopeless cases.

I am indebted to the following physicians for permission to report Case I. This case was under the direct charge of Drs. O. H. Timmins and John B. Herff. Consultants, but in daily attendance, were Dr. Herbert Hill, Internist, Dr. C. F. Lehmann, Dermatologist, Drs. Charles Boehs and W. D. Hicks, Ophthalmologists, Drs. Max Johnson, C. E. Scull, Dudley Jackson, general surgeons and Drs. R. R. Ross and W. H. Heck, Urologists. I was intrusted with the laboratory studies, the preparation of the bacteriophage, and the direction of its use. Case 2 was under the care of Dr. Dudley Jackson who gave permission to include it in this paper.

REFERENCES

1. Eagleton, Wells P.: Cavernous Sinus Thrombophlebitis, Macmillan Company, 196 p., 1926.
2. Smith, Dorland: Cavernous Sinus Thrombosis With Notes of Five Cases, Arch. Ophth. **47:** 482-496, 1918.
3. Dixon, O. Jason: The Pathological Examination in Cavernous Sinus Thrombosis as a Guide to the Diagnosis, Prognosis and Treatment, J. A. M. A. **87:** 1088, 1926.
4. Dwight, E. W., and Germain, H. H.: Thrombosis of the Cavernous Sinus; With Report of Four Cases, Including One Cranial Operation, Boston M. & S. J. **146:** 456, 1902.
5. Babbitt, James A.: The Diagnostic Problem in Orbital Cellulitis, Ann. Otol., Rhinol. & Laryngol. **39:** 444, 1930.
6. Crutchfield, E. D., and Stout, B. F.: Treatment of Staphylococcic Infections of the Skin by the Bacteriophage, Arch. Dermat. & Syph. **22:** 1010, 1930.
7. Lowenstein, Paul S.: Staphylococcus Septicemia, Am. J. M. Sc. **181:** 196, 1931.
8. Rice, Thurman B.: The Use of Bacteriophage Filtrates in the Treatment of Suppurative Conditions (Report of 300 Cases), Am. J. M. Sc. **179:** 345, 1930.
9. d'Herelle, F. D.: The Bacteriophage and Its Clinical Application, Baillière, Tindall & Cox, 243 p., 1930.
10. Bruynoghe, R., and Maisin, J.: Eassais de Thérapeutique au Moyen du Bactériophage du Staphylocoque, Compt. rend. Soc. de biol. **85:** 1120, 1921.
11. Gratia, A.: La Lyse Transmissible du Staphylocoque, Sa Production; ses Applications Therapeutiques, Compt. rend. Soc. de biol. **86:** 276, 1922.
12. Gougerat and Peyre, E.: Le Bactériophage dans le Traitement des Affections Cutanées, Compt. rend. Soc. de biol. **91:** 452, 1924.

13. Hauduroy, Paul: Le Traitement des Infections a Staphylocoques parle Bactériophage de d'Herelle, Presse méd. **34**: 1195, 1926.
14. Grenet, H., and Isaac, Georges: Quelques Essais Thérapeutiques a l'aide du Bactériophage de d'Herelle, Presse méd. **36**: 1089, 1928.
15. Bazy, Louis: Traitement des Infections Chirurgicales a Staphylocoques par le Bactériophage anti-Staphylococcique, Compt. rend. Soc. de biol. **92**: 485, 1925.
16. Lingeman, Byron N.: Furunculosis of External Auditory Meatus, J. Indiana M. A. **22**: 270, 1929.
17. Raiga, A.: Traitement des Furoncles Anthrax par le Bactériophage d'Herelle, Presse méd. **37**: 187, 1929; Traitement, par le Bactériophage de d'Herelle, des Panaris et des plaies infectées des doigts et de la main, Progrès méd. **44**: 415, 1929.
18. Larkum, N. W.: Bacteriophage Treatment of Staphylococcus Infections, J. Infect. Dis. **45**: 34, 1929.
19. Alderson, Harry E.: The Bacteriophage in Pyogenic Infection of the Skin, Arch. Dermat. & Syph. **21**: 197, 1930.
20. Nelson, A. R.: The Effect of Bacteriophage Upon the Phenomena of Leucocytosis and Phagocytosis, J. Immunol. **15**: 43, 1928.
21. Smith, George H.: Bacteriophage and Phagocytosis, J. Immunol. **15**: 125, 1928.
22. Gohs, Waldemar, and Jacobsohn, Irene: Ueber die Lysoresistenz und Lysogenität der sekundären Kulturen beim d'Herelleschen Phanomen, Ztschr. f. Immunitätsforsch. u. exper. Therap. **49**: 17, 1927.
23. Arnold, Lloyd, and Weiss, Emil: Prophylactic and Therapeutic Possibilities of the Twort-d'Herelle's Bacteriophage, J. Lab. & Clin. Med. **12**: 20, 1926-27.

THE GONOCOCCUS COMPLEMENT-FIXATION TEST IN SYNOVIAL FLUID

By David H. Kling, M.D.,* and Julius Pinkus, M.D.,
New York City, N. Y.

THE value of the complement-fixation test in the blood serum for diagnosis of gonorrheal arthritis is recognized by all investigators. Our study presents this reaction in the synovial fluids of arthritis of different etiology and stage.

TECHNIC

We have used the polyvalent antigen manufactured by the Lederle Laboratories according to the principles of Teague and Torrey. Experiments with various amounts demonstrated that 0.1 c.c. of synovial fluid was the optimum to secure specific reactions. Inactivation of the synovial fluid was found to be necessary to prevent anticomplementary reaction. The fluids must be perfectly clear; it has been found that even slight turbidity or hemolysis have interfered with the results. The following routine was used:

The synovial effusion was centrifuged at high speed until the clear fluid is separated from the sediment. Coagula were detached from the walls of the test tubes by means of a glass rod. The clear fluid is inactivated from fifteen to thirty minutes in a water-bath at 56° C. To 0.1 c.c. of synovial fluid in a small test tube there was added 0.1 c.c. of 1:10 dilution of gonococcus antigen, and the amount of complement determined by titration, together with 0.5 c.c. of normal saline. The tubes were then shaken and incubated in the water-

*Frederick Brown Orthopedic Research Fellow.

Received for publication, March 20, 1931.

bath at 37° C. for one hour, after which they were placed in the ice box for three hours. Five-tenths c.c. of 5 per cent suspension of sheep cells and 1 unit of ambocepter were added. The whole was incubated for one hour and readings were made. Controls, positive, negative and hemolytic, as well as anticomplementary, were run with each test. Complete fixation is marked four-plus; three-, two-, and one-plus signify degrees of partial fixation; complete hemolysis is marked negative. Fluids should be preserved in the ice box and tests carried out as soon as possible.

ANALYSIS OF THE MATERIAL

The reaction was carried out on 121 cases. Fifteen fluids were aspirated from traumatic and 106 (87.6 per cent) from inflammatory effusions. Of the latter 36 (33.9 per cent) were from acute cases and 70 (66.1 per cent) were from chronic conditions (duration over six months). Of the 106 inflammatory fluids, 95 (89.6 per cent) gave a negative reaction and 11 (10.4 per cent) a positive reaction. Of these 11 positives, 7 were aspirated from knee joints, 2 from elbows, 1 from the shoulder and 1 from the ankle. The reaction was four-plus in 6 fluids, three-plus in 1 fluid, two-plus in 3, and one-plus in 1 fluid. Of the 36 acute effusions, 8 (22.2 per cent) gave positive results; of the 70 chronic cases, 3 (4.3 per cent) gave positive reactions.

SPECIFICITY OF REACTION

a. The Wassermann reaction was carried out simultaneously in all, and was positive in 16 fluids. Of these, 13 cases had a negative gonococcus complement-fixation test. In 3 fluids both reactions were strongly positive. The history in 2 cases showed an old syphilitic and a recent gonorrheal infection.

CASE 1.—(No. 4 on Table I). J. McD., twenty-three years old. Contracted gonorrheal urethritis three months previously, followed by epididimitis. Two months ago pain in left wrist and toes. At the time of his admission to the clinic he had a swelling of the left wrist and right knee joints. Sixty c.c. of turbid fluid containing 26,100 white cells per c.mm. with 86 per cent polymorphonuclear leucocytes were aspirated. The Wassermann and gonorrheal fixation tests were four-plus in both blood and fluid. Culture of the synovial fluid gave no growth. However, gonococci were found in a smear of the urethral discharge.

CASE 2.—(No. 1 on Table I.) C. B., twenty-nine years old. Contracted gonorrhea four weeks previously and for two weeks the right ankle had been swollen and tender. Three c.c. of a turbid fluid containing numerous polymorphonuclear leucocytes were aspirated. The Wassermann and gonococcus complement-fixation tests gave a four-plus in the fluid and blood serum. Culture of the synovial fluid was negative but gonococci were found in direct smear from the synovial fluid.

CASE 3.—(No. 6 on Table I.) L. W., colored female, thirty-one years old. Pain and swelling of wrists and sterno-clavicular joints and effusion of both knees for about a year. Ptosis of the right eyelid. The aspirated fluid was turbid with a white count of 1,800 per c.mm. and 46 per cent polymorphonuclear leucocytes. Wassermann and gonococcus fixation tests in the fluid were four-plus; Wassermann in the blood was four-plus; gonococcus in the blood was two-plus.

b. Thirteen fluids were inoculated into guinea pigs, of these 4 developed tuberculosis. None of the fluids gave a positive gonococcus complement-fixation test. However, one fluid obtained at operation showed a two-plus

gonococcus reaction while the histologic examination of the synovial membrane revealed tubercles.

TABLE I*

NO.	NAME	DURATION GONORRHEA	DURATION ARTHRITIS	UROGENITAL GONORRHEA	GONOCOCCUS COMPLEMENT FIXATION		REMARKS
					SYNOVIAL FLUID	BLOOD	
1	C.B.	4 wk.	2 wk.	Urethral epididymitis	++++	++++	Wassermann ++++; gonorrhea positive in sediment of synovial fluid and discharge of urethra
2	O.K.	8 wk.	5 wk.	Urethral and prostatitis	++++	Not done	
3	D.K.	11 wk.	1 wk.	Prostatitis	++++	++++	
4	J.McD.	15 wk.	8 wk.	Epididymitis	++++	++++	Wassermann ++++; gonorrhea positive in discharge
5	E.R.	2 yr.	6 wk.	Prostatitis	++++	++++	
6	L.W.	?	1 yr.	?	++++	++	Wassermann ++++
7	M.A.	2½ yr.	3 wk.	?	+++	—	First attack 2 years ago
8	T.	?	2 wk.	?	++	Not done	
9	S.W.	?	1 yr.	?	++	Not done	
10	G.F.	3 yr.	2 yr.	?	++	—	Tubercular synovitis
11	H.T.	4 mo.	3 wk.	Prostatitis	+	—	

*Numbers 6 and 9 are female, all others are male. All cultures were negative.

CASE 4.—(No. 10 on Table I.) G. F., twenty-six years old. Five years before admission he fell down and injured his left knee. Several years ago he became infected with gonorrhea. For two years he suffered with swelling and pain of the left knee joint. There was effusion and a small tumor outside of the joint which was taken for a cyst of the external semilunar cartilage. A synovectomy was done and the membrane was found to be hypertrophic and with necrotic villi in some places. Sections showed numerous tubercules. Twenty c.c. of fluid was recovered with some red cells. Gonococcus fixation was two-plus and Wassermann was negative in the fluid; while in the blood they were both negative.

c. In 79 fluids, cultures were made and 19 were positive for different strains of staphylococci, streptococci, and diphtheroids. None of these fluids gave positive gonococcus reactions.

Check-up examinations on reaspiration of a number of fluids gave identical results.

It is therefore concluded that with the eventual exception of a weak positive reaction in one case of tubercular arthritis, the presence of specific or nonspecific infection did not interfere with the specificity of the gonococcus complement fixation in synovial fluids.

CLINICAL CONSIDERATIONS

Of the 6 patients with complete gonococcus complement fixation in the synovial fluids, 5 were males between the ages of twenty-three and thirty-six years. All had some urethral discharge. Prostatitis was present in 3 cases and epididymitis in two. The onset of arthritis was from one to eight weeks previous to examination. In 4 cases several joints were involved, the knee joint being chiefly affected. One patient suffered from an arthritis of the ankle joint only. The clinical picture was an acute infectious arthritis with

pain, peri-articular swelling, limitation of motion, and effusion. The synovial fluid was turbid, with a high cell count and contained polymorphonuclear leucocytes from 60 per cent to 90 per cent. In 4 of these cases, the gonococcus complement-fixation test in blood serum was carried out and gave a four-plus reaction. The cultures were negative in all cases for gonorrhea and other organisms. In one case gonococci were demonstrated in the smear from the sediment of the synovial fluid. Of the 5 cases with partial complement fixation one gave a three-plus in the synovial fluid, while the blood serum was negative.

CASE 5.—(No. 7 on Table I.) M. A., twenty-seven years old. He had gonorrheal urethritis two and one-half years previously and an arthritis of both knees two years ago. For three weeks he had had effusion in both knees and transitory pains and swelling in feet, ankles, and right sternoclavicular joint. The fluid aspirated from the knee joint was cloudy and contained 92 per cent polymorphonuclear leucocytes; the cultures were sterile after eight weeks. There was no involvement of the heart; no evidence of still persisting gonorrheal infection. Salicylates had litttle effect and improvement followed after injection of the filtrate from the synovial fluid.

Of the three patients who gave a two-plus gonococcus complement fixation in the fluids, one (Case 4) was proved to be tubercular arthritis of the right knee. The second patient, a man thirty-eight years old, suffered from a subacromial bursitis for two weeks previously. The effusion was turbid and contained 75 per cent polymorphonuclear leucocytes. He did not admit gonorrheal infection; no urologic examination was carried out and blood serum was not tested. The third case was a woman of twenty-nine years, who gave the history of an acute polyarthritis three years before. For three weeks she has had a recurrence with pain in the feet and wrists and effusion in the right elbow joint. No record of gonorrheal infection was obtained and blood serum was not tested.

One patient gave a one-plus positive complement fixation in the synovial fluid while the blood serum was negative.

CASE 6.—(No. 11 on Table I.) H. T., thirty-six years old. Four months previously he had a gonorrheal infection. Seven weeks ago there was a sudden onset of pain, swelling and effusion in the right knee, with an increase in local and general temperature. The synovial fluid was cloudy and contained 98 per cent of polymorphonuclear leucocytes. The prostatic gland was swollen and tender. The secretion was purulent. No gonococci were found. An improvement was effected by intravenous injections of typhoid vaccine.

COMPARISON OF GONOCOCCUS COMPLEMENT OF BLOOD SERUM WITH SYNOVIAL FLUID

The complement-fixation test was carried out simultaneously in the blood serum in 8 cases. In 4 cases the reaction was four-plus in agreement with the results in the synovial fluid. These were cases of acute arthritis with other evidence of still persisting gonorrheal infection and complications. In one case of chronic infectious arthritis, the reaction was two-plus in the serum while the synovial fluid gave a four-plus. In three cases with partial fixation in the synovial fluid the blood serum was negative. Of these, one patient had acute arthritis of the knee and a urethritis and prostatitis. Two had old gonorrheal infections but no evidence of recent activity. One of these was histologically proved to be a case of tubercular synovitis. The synovial

fluid gave a higher percentage of positive reactions and a stronger reaction than the blood serum. Some evidence can be adducted that this is due to a higher concentration of antibodies in the synovial fluid rather than to interference by unspecific substances. In 95 fluids, including 36 cases of acute inflammatory arthritis, the reaction was negative. A number of these cases had a history of gonorrheal infection. The patients with strong positive reactions and at least one with a weak positive reaction gave other evidence of active gonorrheal infection. On this basis, we must consider that the partial gonococcus complement fixation in the synovial fluid alone may indicate that gonorrhea is at least one of the factors involved in the etiology of the arthritic condition. However, the material is too limited and further studies are needed to definitely estimate the value of partial gonococcus complement fixation in the synovial fluid.

CONCLUSIONS AND SUMMARY

1. The technic of the gonococcus complement-fixation test in the synovial fluid is outlined and the results in 106 cases of acute and chronic inflammatory arthritis are reported.

2. The reaction was found to be positive in 11 cases (10.4 per cent). The presence of other infections does not interfere markedly with the specificity of the reaction.

3. In 7 cases other evidences of active gonorrheal infection were found but in 4 cases this reaction was the only evidence of the gonorrheal etiology.

4. In 4 cases both blood and synovial fluid gave complete gonorrheal complement-fixation tests. In one case the blood gave only a weak positive reaction while the synovial fluid gave complete positive reaction. In 3 cases the synovial fluid gave partial complement fixation and the blood was negative.

5. The strong positive reaction in the synovial fluid is therefore considered a valid proof of the gonorrheal etiology of the arthritis and more conclusive than the reaction in the blood serum where it only indicates the presence of an active focus somewhere in the body.

6. The significance of partial complement fixation in the synovial fluid needs further investigation.

7. It is recommended on the basis of these findings to carry out the gonococcus complement-fixation tests as a routine examination in all synovial fluids.

NOTE: The material for this study was derived from the orthopedic services of Drs. Harry Finkelstein, Herman Frauenthal, Samuel Kleinberg, and Leon Mayer to whom we express our appreciation.

1919 MADISON AVENUE.

THE EFFECTS OF ULTRAVIOLET IRRADIATION ON THE REDUCING POWER OF BLOOD*†

By L. M. Dillman, Chicago, Ill.

KOCH and Reed,[17] irradiating, with a carbon arc lamp blood flowing through a quartz tube inserted into the carotid artery in etherized dogs, reported increased uric acid values. This increase, an average of 38 per cent in twenty-one dogs, was determined by the phosphotungstic acid colorimetric method of Folin and Wu as modified by Koch.[17] It was recognized at that time that there was no actual increase of uric acid in the blood, but that the reaction was due to other reducing substances.

The experiment was repeated by Reed and Barnard[21] with somewhat varying results, sufficient to justify a reinvestigation to determine, if possible, the interfering substances.

EXPERIMENTAL PROCEDURE

Forty to fifty c.c. of blood were drawn from a dog, using $Na_2C_2O_4$ as an anticoagulant. This sample was divided into three portions. The first portion was analyzed immediately. The second was placed in a fused quartz flask 5 mm. in thickness and irradiated with a Kromayer quartz-mercury vapor lamp at a distance of one inch (at this distance there was uniform diffusion throughout the flask) at room temperature; samples were analyzed at thirty, sixty, and ninety minute intervals. The third portion from the original sample was placed in a glass flask similar to the one used in irradiating the blood, set in an adjoining room as a control, and analyzed after ninety minutes. Analyses were made directly upon the blood filtrates according to Folin's[10] method. The results of this experiment are shown in Table I. An increase in reducing power in irradiated blood is noticeable in only one in-

Table I

DOG	ORIGINAL	MINUTES			IRRADIATED	CONTROL
		30	60	90		
I	1.70	2.16		2.20	2.15	1.60
II	1.39	1.34		1.14	1.28	1.42
III	0.66				0.80	0.72
IV	0.75			0.90	0.85	1.00
V	2.81				2.64	
VI	0.97				0.86	
VII	0.86				0.89	
VIII	1.47				1.53	
IX	1.54				1.51	

Results are in mg. uric acid per 100 c.c. blood. For obvious reasons some of the samples were not analyzed.

*From the Department of Physiology, University of Illinois, College of Medicine, Chicago. Received for publication, April 21, 1931.

†This investigation was financed in part by grants from the Graduate School; from the Phi Rho Sigma Medical Fraternity; and from the Committee on Scientific Investigation, American Medical Association.

stance, Number K. In this case, however, the colorimetric readings were as 16/22 which introducees an error large enough to account for the results. The other experiments show no increase whatever, outside of the percentage of error.

In view of the fact that Koch and Reed had used a modification of Folin's uric acid method, it was thought that perhaps this might account for failure to duplicate their results. In the last three experiments VII, VIII, and IX, samples were also analyzed by Koch's modification of Folin's method which is essentially the same except that 5 c.c. of 4 per cent NaCN in N/7 lithium hydroxide is substituted for 2 c.c. of 15 per cent NaCN in N/10 NaOH. No increased reduction was noted though there was a difference in color produced. This is indicated in Table II.

Ten c.c. portions of the above samples were added to 7 c.c. of Silver lactate, the mixture centrifuged, the supernatent liquid poured off, and the precipitate washed with 10 per cent NaCl in N/10 HCl. This washing gave no color deep enough to read with either set of reagents.

TABLE II

DOG	ORIGINAL		90 MIN. IRRADIATION	
	Koch	Folin	Koch	Folin
VII	1.2	0.86	1.13	0.89
VIII	1.41	1.47	1.49	1.53
IX	1.12	1.54	1.12	1.51

Results in mg. uric acid 100 c.c. blood.

As indicated in Tables I and II the results of Koch and Reed in vivo cannot be produced in vitro. This may be due to a mechanical effect difficult to obviate: in irradiating whole blood the proteins coagulate in a film on the side of the flask nearer the lamp which may prevent penetration. There is also a tendency for the corpuscles to settle out, thus reducing the surface of contact between corpuscles and plasms. In order to determine the importance of this effect, the following experiment was made.

Uric acid, in the presence of ultraviolet light, loses its power to reduce phosphotungstic acid, as indicated:

TABLE III

SOLUTION	AFTER 90 MIN. IRRADIATION	PER CENT LOSS
1 92.8	90.6	2.3
2 19.28	11.9	38.2
3 0.96	0.179	81.3

Mg. uric acid/100 c.c.

Solution 1 dissolved in Na_2CO_3.

Solution 2 and 3 dissolved in Li_2CO_3.

Exactly 1.0 mg. of uric acid (Folin Standard) was added to 40 c.c. of dog blood and thoroughly mixed. A sample was analyzed immediately and the remainder was divided into two portions, one of which was irradiated ninety minutes, the other was used as a control. Proteins were precipitated with tungstic acid and 10 c.c. portions of the filtrate were analyzed for uric acid by Folin's isolation procedure.[10]

TABLE IV

ADDED	RECOVERED	AFTER IRRADIATING 90 MIN.	CONTROL
2.5	1.61	0.38	1.57
2.5	1.18	0.94	1.25

Mg. uric acid/100 c.c.

In both cases a film was formed on the side of the flask and the loss of uric acid corresponds roughly to the penetration. The amount of recovered uric acid is about the same as reported for human blood and for sheep blood.[4, 12]

The newer method of Folin,[11] however, recovers uric acid quantitatively from sheep or human blood; we may add that the method works as well for dog blood.

The above experiment was repeated using this newer method of Folin on unlaked blood extract on the only blood available at the time from a pregnant bitch. In this case, however, of the 4 mg. per cent added, the same amount was recovered immediately, ninety minutes later, and after another ninety minutes' interval, and also after ninety minutes' irradiation. The film on the flask came off as a coating.

To determine the relation of glucose, aqueous solutions of glucose (Merck's cp dried twenty-four hours at 85°) were irradiated under conditions as given above. After irradiation the samples were analyzed with Folin uric acid reagents[10] and matched against a standard of uric acid. In using this standard, interference of glucose in blood uric acid determinations can be seen at a glance; but, more important, uric acid presented a constant standard for determining a change in reducing power of the glucose solution.

The results of Table V seem to indicate the inconstancy of this method while dilute solutions of substances other than uric acid, the variation being inversely as the color produced. Similar variations are shown in Tables I and II.

TABLE V

GLUCOSE PER CENT	MINUTES IRRADIATED	ANALYSIS (1) MG. U. A. PER 100 C.C.	ANALYSIS (2)	ANALYSIS (3)	GREATEST DIFF.	PER CENT DIFF.
2.00	0	1.10	0.90		0.20	20
1.75	0	0.77	0.98		0.21	23
1.50	0	0.49	0.56	0.42	0.14	28
	30	0.54				
	60	0.57				
	90	0.45	0.54	0.62	0.17	31
1.25	0	0.49	0.52	0.40	0.12	25
	30	0.58				
	60	0.58				
	90	0.54				
1.00	0	0.29	0.47, 0.29	0.38	0.18	36
	30	0.48	0.51		0.03	3
	60	0.45				
0.8		0.31				
0.6		0.20				
0.4		0.16				
0.1		0.05				
		0.05				

The interference of glucose in the analysis of normal blood filtrates for uric acid is practically none; even in cases of diabetes with a blood sugar of 1 per cent the interference would be less than 0.5 mg./100 c.c. of blood if a "direct" method was used. This interference is practically nil in view of the increased uric acid in diabetic patients.

DISCUSSION

It is important to note that the so-called uric acid value by no means represents uric acid. It is, to be exact, the value obtained by reducing phosphotungstic or arsenophosphotungstic acid in the presence of alkali and cyanide, compared with a standard of uric acid. The color produced obeys Beer's Law only within very narrow limits, so the blood filtrate and the standard should be very nearly the same concentration or sizable errors are introduced in finally matching the colors. A number of substances also reduce the reagent, or otherwise influence the reaction. Grigaut[13] after trying some hundred compounds that may be present in protein-free blood filtrates, reports that uric acid, alloxane, and alloxantine alone reduce phosphotungstic acid but polyphenol medicants influence the reaction. Incidentally this observer finds that glucose does not influence the reaction.

The findings of Grigaut, could not explain the discrepancies, noted by workers in this country, between the values given by "direct" and "indirect" uric acid methods.[6, 7, 18] These discrepancies indicated the presence of a substance, or substances, that interfered with uric acid determination when made directly upon the protein-free blood filtrates. Especially was this true of animal bloods in which the actual uric acid content is thought to be 0.1 mg. per 100 c.c. or even less. As a result of this, Benedict, Newton and Behre[3] isolated a compound from pig blood which they called thiasine. Independently, and at about the same time, Hunter and Eagles[15] isolated a compound from pig blood having very similar properties, which they called sympectothion. It was later shown by Newton, Benedict, and Dakin[19] that thiasine was identical with ergothionine isolated from ergot of rye and with the sympectothion of Hunter and Eagles. For this compound, the betaine thiolhistidine, Benedict proposes the name thioneine for the one of animal origin. The work of Newton, Benedict, and Dakin was verified independently by Eagles and Johnson.[9]

When Blumer, Eagles and Hunter[7] were isolating Substance X, or sympectothion, or thioneine they suspected the presence of another substance which also reduced phosphotungstic acid. They later isolated this substance which proved to be glutathione.[16]

Thus within the last few years two unsuspected substances have been isolated from blood which influences "direct" uric acid determination, and it is indicated that there are others. Rockwood, Turner, and Pfiffner[20] report the presence of another interfering substance after acid hydrolysis of tungstic acid blood and tissue filtrates. They call this Substance Z. Behr and Benedict,[1] in determining the thioneine content of human blood conclude that there must be one other substance (other than uric acid, thioneine, or glutathione)

which yields color with uric acid reagents. When viewed in this light the findings of Koch and Reed take on a new significance.

The results of Koch and Reed in vivo cannot be produced in vitro; the reason is partly, but not wholly, mechanical. Uric acid disappears rather rapidly when irradiated in mild alkaline solution and analyses seem to indicate that it disappears from blood on irradiation provided there is penetration.

No attempt is made to determine the relation of glutathione or thioneine to this reaction because methods for analyzing these substances are not well enough established, nor could we, at present, observe them in pure solution.

CONCLUSIONS

1. The increase of reducing power of blood irradiated in vivo cannot be duplicated by irradiation in vitro.

2. Uric acid under the influence of ultraviolet irradiation loses its power to reduce phosphotungstic acid.

REFERENCES

1. Behre, F. A., and Benedict, S. R.: The Occurrence and Determination of Thioneine (Ergothioneine) in Human Blood, J. Biol. Chem. **82**: 11, 1929.
2. Benedict, S. R., and Newton, E. B.: Studies on the Non-Sugar Reducing Substances of the Blood and Urine. I. Glutathione and Thioneine in Blood, J. Biol. Chem. **83**: 361, 1929.
3. Benedict, S. R., Newton, E. B., and Behre, J. A.: A New Sulphur-Containing Compound (Thiasine) in the Blood, J. Biol. Chem. **67**: 267, 1926.
4. Benedict, S. R., and Newton, E. B.: The Use of Molybolic Acid as a Precipitant for Blood Proteins, J. Biol. Chem. **82**: 5, 1929.
5. Benedict, S. R.: Determination of Uric Acid in Blood, J. Biol. Chem. **51**: 187, 1922; Determination of Uric Acid, **54**: 233, 1922.
6. Benedict, S. R.: The Determination of Uric Acid in the Blood, J. Biol. Chem. **64**: 215, 1925.
7. Blumer, J. M. R., Eagles, B. A., and Hunter, G.: Uric Acid Determination in Blood, J. Biol. Chem. **63**: 17, 1925.
8. Brown, H., and Raiziss, G. W.: The Estimation of Uric Acid in the Blood, J. LAB. & CLIN. MED. **8**: 129, 1922-23.
9. Eagles, B. A., and Johnson, Treat B.: The Biochemistry of Sulphur. I. The Identity of Ergothioneine From Ergot of Rye With Sympectothion and Thiasine From Pig's Blood, J. Am. Chem. Soc. **49**: 575, 1927.
10. Folin, O.: A Revision of the Method for Determining Uric Acid, J. Biol. Chem. **54**: 153, 1922.
11. Folin, O.: An Improved Method for the Determination of Uric Acid in Blood, J. Biol. Chem. **86**: 179, 1930.
12. Folin, O.: A System of Blood Analysis, J. Biol. Chem. **38**: 81, 1919.
13. Grigaut, A.: Specificite 'de la reaction phosphotungstique pour le dosage de l'acide urique. C. R. Soc. Biol. **84**: 632, 1921.
14. Hunter, G., and Eagles, B. A.: The Isolation From Blood of a Hitherto Unknown Substance, and Its Bearing on Present Methods for the Estimation of Uric Acid, J. Biol. Chem. **65**: 623, 1925.
15. Hunter, G., and Eagles, H. H.: Non-sulfur Compounds of Blood. I. Sympectothion, J. Biol. Chem. **72**: 123, 1927.
16. Hunter, G., and Eagles, B. A.: Non-sulfur Compounds of Blood. II. Glutathione, J. Biol. Chem. **72**: 133, 1927.
17. Koch, F. C., and Reed, C. I.: Studies on the Physiological Action of Light. V. Increase in Uric Acid in Blood Irradiated Directly, Am. J. Physiol. **75**: 351, 1925.
18. Lennox, W. G., and O'Connor, M. F.: Measurement of Uric Acid in Blood by Various Methods, J. LAB. & CLIN. MED. **10**: 99, 1924.
19. Newton, E. B., Benedict, S. R., and Dakin, H. D.: On Thiasine, Its Structure and Identification With Ergothioneine, J. Biol. Chem. **72**: 367, 1927.
20. Rockwood, E. W., Turner, R. G., and Pfiffner: A Previously Undetected Constituent of Blood, J. Biol. Chem. **83**: 289, 1929.
21. Reed, C. I., and Barnard, R. D.: Studies on the Physiological Action of Light. VIII. An Attempt to Characterize the Substance Giving Increased Uric Acid Values After Irradiation of Blood, Am. J. Physiol. **93**: 146, 1930.

THE RELATION OF THE P_H REACTION OF URINE TO THE ANTISEPTIC ACTION OF MALLOPHENE IN VITRO*

By Russell D. Herrold, M.D., and Earl E. Ewert, M.D., Chicago, Ill.

SEVERAL urinary antiseptics of the pyridine type have been placed upon the market during the last few years. These drugs are administered orally and are known to be excreted selectively by the kidneys so that practically all of the ingested drug is eliminated in the urine. We have studied one of these drugs known as mallophene (beta-phenyl-azo-alpha-alpha-diamino-pyridine hydrochloride) quite extensively during the last several months. Clinical observations have been made, but more particular attention has been given experimentally to the relation of the hydrogen-ion concentration of the urine to the bactericidal and bacteriostatic action against staphylococci and colon bacilli in the presence of various dilutions of mallophene.

Mallophene has many of the characteristics of an ideal antiseptic since it seldom produces any disturbance of the intestinal tract, and can be given in therapeutic dosage over a long period of time without any apparent irritation of the kidneys. Such a dye compound is perhaps more penetrating than many other antiseptics. While the amount excreted in the urine is not completely bactericidal for the colon bacillus, it is sufficiently bacteriostatic that when used over a fairly long period of time it seems to produce bacteriologic cure in many of the acute uncomplicated types of infection. When combined with pelvic lavage and drainage in the chronic type of infection it has given better results clinically in my experience than any other urinary antiseptic previously used. In the typical acute case it is advisable to give one tablet three times a day for a few days to determine tolerance for the drug, and then the dosage is increased to two tablets two or three times a day until the amount of pus and number of bacteria in the urine are definitely decreased. Later the dosage is decreased to two tablets once daily until sterile cultures have been obtained. It is advisable to continue two tablets every other day for a short period after bacteriologic cure has been obtained to decrease later exacerbations.

A few clinical papers have been published during the last several years in which stress has been made of the importance of acidity as a factor in the successful management of urinary infections, but its necessity is more generally considered to be a means whereby formalin is liberated from hexamethylenamine. More recently Hiller and Stamler[1] have noted that mercurochrome is more bactericidal in acid than in alkaline mediums by laboratory tests. There was an exception in a short range on the acid side.

Preliminary observations were made by the addition of varying quantities of 2 per cent acid sodium phosphate and 2 per cent alkaline sodium phosphate to the same specimen of urine. Dilutions of mallophene were then added and

*From the Department of Urology, College of Medicine of the University of Illinois.

inoculations made with colon bacilli. Subcultures were made after three days on plain nutrient agar. The results are tabulated in Table I. Hydrogen-ion concentrations were not made in this preliminary observation, but the antiseptic action of mallophene was greater in the urine specimens containing the acid sodium phosphate. There was a slight inhibition of the growth of the colon bacilli in the urine specimen containing acid sodium phosphate alone as compared to the urine with alkaline sodium phosphate alone.

Further observations were then made to determine the comparative bactericidal action at known hydrogen-ion concentrations. Urine was used as the medium for addition of various dilutions of mallophene since it seemed logical that such observations would be more comparable to later experiments in vivo. The technic served as a means for the determination of the bacteriostatic action as emphasized in the "growth-restraining" test of Leonard[2] since urine serves as a good medium for the growth of colon bacilli and staphylococci. In addition the transfer test at the end of three or four days' incubation gave a consistent bactericidal result for comparative purposes. The results tabulated in Table II

TABLE I

IN VITRO CULTURAL RESULTS OF B. COLI ADDED TO ACID OR ALKALINE SODIUM PHOSPHATE IN URINE WITH MALLOPHENE

DILUTION OF MALLOPHENE	90 C.C. URINE 88.5 C.C. 2% ACID SODIUM PHOSPHATE	90 C.C. URINE 28.5 C.C. 2% ACID SODIUM PHOSPHATE	90 C.C. URINE 28.5 C.C. 2% ALKALINE SODIUM PHOSPHATE	90 C.C. URINE 88.5 C.C. 2% ALKALINE SODIUM PHOSPHATE
1–2000	0	0	3+	3+
1–4000	0	0	4+	4+
1–8000	0	0	4+	4+
Control urine without mallophene	3+	3+	4+	4+

indicate that there is a very definite increase in the bactericidal action for colon bacilli and staphylococci at the acid range of P_H 5.2 and P_H 5.8 as compared to acid range P_H 6.6 and alkaline range P_H 7.4. In this experiment a single urine was used and the specimens voided during the same day starting with the acid P_H 5.2. Then alkalies were taken by mouth, and succeeding specimens were collected to obtain urines with the higher hydrogen-ion concentration as indicated in Table II so that the difference in hydrogen-ion concentration was the only variation in the medium with the same mallophene dilution. If these results prove consistent with similar observations in vitro it would seem worth while to consider the routine hydrogen-ion determination as a means of obtaining the maximum efficiency of urinary antiseptics in the management of urinary

TABLE II

MALLOPHENE ADDED TO URINE IN VITRO

P_H	COLI				STAPHYLOCOCCI			
	1000	2000	4000	6000	1000	2000	4000	6000
5.2	0	0	0	2+	0	0	0	1+
5.8	0	0	0	3+	0	0	0	2+
6.6	3+	3+	4+	4+	0	2+	3+	3+
7.4	4+	4+	4+	4+	2+	2+	3+	4+

infections. We have noted that the range of hydrogen-ion concentration may be estimated roughly by boiling a specimen of urine for five minutes in a test tube. If clouding occurs after heat, the P_H is higher than 6.0, and antiseptics in such urine are of less value, but if the urine remains clear, the acidity is below P_H 6.0 and within or bordering on the optimum range.

It was interesting to note the average hydrogen-ion concentration of the urines in a series of 75 patients. These specimens were tested immediately after voiding, and without the influence of urinary antiseptics or urinary acidifying agents. The results are tabulated in Table III and are divided into two classes, private and clinic patients. It may be noted without any definite significance that a higher percentage of private patients in this small series of cases had urines with a hydrogen-ion concentration within the range that we would not consider to be the optimum for urinary antiseptics, particularly mallophene, as indicated by the above bactericidal tests. If this comparative increase in the higher hydrogen-ion concentration with private patients should prove consistent with a larger series of cases, it might be explained because of the difference in time of observation. The private patients were seen in the afternoon or evening while most of the clinic patients were seen during the morning hours. Again there may be some variation as the result of the dietary differences of the two classes of patients.

TABLE III

ROUTINE P_H OF PATIENTS ON EXAMINATION

TYPE OF PATIENTS	NUMBER WITH P_H 4.8—5.2 INCLUSIVE	NUMBER WITH P_H 5.3—5.7 INCLUSIVE	NUMBER WITH P_H 5.8—6.2 INCLUSIVE	NUMBER WITH P_H 6.3—6.7 INCLUSIVE	NUMBER WITH P_H 6.8—7.2 INCLUSIVE
Clinic	20	16	6	4	1
Private	7	5	7	4	5

DISCUSSION

It is evident that if the hydrogen-ion concentration influences the optimum range of action of urinary antiseptics in vivo as it clearly does in experiments in vitro, more importance should be given to such observations in urinary infections, and particularly to the time relationship between the administration of the urinary antiseptic and the urinary acidifier. Fiske[3] states that an alkaline tide occurs regularly after meals. We have made comparative observations of three commonly used acidifying drugs, acid sodium phosphate, sodium benzoate and ammonium chloride. While the observations have not been sufficiently extensive to draw definite conclusions, we have noted that ammonium chloride is less likely to be followed by a temporary change toward the alkaline range than is acid sodium phosphate and sodium benzoate. In one instance a dose of four grams of acid sodium phosphate was given and a specimen two hours later had a hydrogen-ion reading of 5.5. Specimens taken at intervals up to six hours indicated a gradual increase in the hydrogen-ion reading so that six hours after the ingestion of acid sodium phosphate the P_H reading was 6.1. It would seem that further observations are necessary on the influence of the size of the dose of the various acidifying drugs.

The impression is gained by the observation of repeated hydrogen-ion determinations at intervals of several days that certain individuals are likely to have regularly, a reaction consistently low on the acid side. Others seem more likely to have reactions consistently high on the acid side and even occasionally alkaline.

CONCLUSIONS

1. Contrary to the usual belief, antiseptics particularly of the pyridine type seem to be more efficient by experiments in vitro when added to urine in the lower acid range of hydrogen-ion concentration than in the higher acid or alkaline range.

2. The routine hydrogen-ion determination in a series of 75 patients indicates that a majority are within the limits of optimum reaction for efficient antiseptic, yet there is a sufficient number in the less efficient range to indicate a necessity for acidifying agents to obtain the greatest efficiency of the urinary antiseptics.

3. This increased efficiency of mallophene in the more acid urines seems to apply equally to colon bacilli and staphylococci as indicated by experiments in vitro.

REFERENCES

1. Hiller, R. I., and Stamler, A. E.: Effect of Hydrogen-ion Concentration on Bactericidal Action of Mercurochrome-220, Soluble on B. coli, J. Urology **22**: 699, 1929.
2. Leonard, G. F.: Limitations of Phenol Coefficient Tests in Determining Germicidal Activities, J. Infect. Dis. **48**: 358, 1931.
3. Fiske, C. H.: "Alkaline Tide" After Meals, J. Biol. Chem. **49**: 163, 1921.

LABORATORY METHODS

THE ESTIMATION OF THE SERUM CAROTIN*

By F. D. White, Ph.D., F.I.C., with the assistance of Ethel M. Gordon,
Winnipeg, Canada

THE yellow pigments designated as lipochromes or carotinoids have been the subjects of many investigations. A very complete bibliography up to 1922 is contained in the monograph by Palmer[1] while more recent work is reviewed in the papers by Boeck and Yater[2] and by Stannus.[3] Our present knowledge of these pigments in connection with human blood serum can be summarized briefly. The pigments carotin and xanthophyll are present in small quantities in the blood of normal individuals, giving a yellow color to the serum, and can be estimated by means of a technic elaborated by Palmer. Under certain conditions, notably in cases of diabetes, the amount of this pigmentation is greatly increased, and it has been shown that this increase is chiefly due to increased quantities of carotin; hence the condition is known as carotinemia, although the more general term, xanthemia, is also used. If the xanthemia is very pronounced it may be accompanied by skin pigmentation or xanthosis, sometimes referred to as pseudoicterus since it resembles the pigmented appearance of jaundice but differs therefrom in that the sclerotics are not involved. The condition of xanthosis has also been observed in nondiabetic individuals, chiefly children, on a diet rich in carrots. Elimination of this vegetable from the diet generally leads to restoration of the normal skin coloration.

As far as is known at present, an increase in the carotin content of the blood has no particular clinical significance, and can be controlled easily by adjustment of the diet. Its possible importance, however, cannot be ignored since we are also in ignorance of the function normally fulfilled in the organism by the carotinoids. The last decade has added considerably to our knowledge of carotin: the close association in plants and vegetables between lipochrome pigments and the fat-soluble accessory food factor, vitamin A, has led to the discovery that minute amounts of purified crystalline carotin (but not xanthophyll) when added to vitamin A-free diets produce effects comparable in every way with those of vitamin A,[4, 5, 6] and that, in effect, carotin can now be regarded as the precursor of that vitamin in the animal organism.[7, 8] Through the researches of Willstätter and Stoll,[9] van den Bergh and his coworkers,[10, 11] Palmer and others, methods are now available for detecting the presence of carotin in serum and estimating it quantitatively. These

*From the Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.
Received for publication, April 20, 1931.

methods primarily depend upon the solubility of carotin in low boiling petroleum ether, and the fact that this solvent will remove carotin quantitatively from its solution in 80 to 90 per cent alcohol. The latter fact is of considerable importance since Palmer has shown that the pigment is apparently in some form of combination with the serum protein, which prevents its direct extraction. Treatment of the serum with alcohol precipitates the proteins and releases the pigment, which can then be extracted. As all other pigments likely to be present, including hemoglobin and bilirubin, remain dissolved in the alcohol, the petroleum ether extract can be removed and compared against a standard solution. This in broad outline is the procedure which is universally adopted, only variations in the details of the technic distinguishing the methods of different investigators.

Since solutions of pure carotin are unstable, for routine determinations an artificial standard is essential, and it has been found that a dilute solution of potassium dichromate gives a color sufficiently close to that of carotin to enable it to be used instead. A perusal of the literature reveals that in the strength of the dichromate standard solution used and its color value in terms of carotin, lie the greatest divergences between the various methods of estimation. The chief difficulty, which has not always been appreciated, is that the relationship between concentration and color intensity in an aqueous solution of potassium dichromate is not the same as exists in a petroleum ether solution of carotin. This is exemplified in Willstätter and Stoll's use of a 0.2 per cent solution of potassium dichromate as standard. They found that a 5×10^{-5} molar solution of carotin (0.00268 per cent) compared in a colorimeter at a depth of 100 mm. against this dichromate solution, gave a color match when the depth of field of the latter was 101 mm. When the carotin solution was placed at 50 and 25 mm. respectively, a color match was obtained with the dichromate solution at the respective depths of 41 and 19 mm. Notwithstanding these divergences of color intensity at different depths of field, colorimetric comparison without adequate corrections has been consistently used in evaluating the degree of carotinemia, and consequently where the values are expressed as actual carotin content, there are inevitably serious discrepancies.

If the depth of field be kept constant then this error is avoided, together with the not inconsiderable error due to the comparison of a very volatile liquid with a nonvolatile one in open colorimeter cups. The following procedure was therefore worked out with the idea of obtaining a routine laboratory method which could be used with a reasonable degree of accuracy. The manipulative procedure is practically that of Palmer as used by Rabinowitch[12] with small amounts of serum, but adapted to give values which can be expressed in terms of carotin. It consists in extracting a mixture of serum, plaster of Paris, and alcohol, with petroleum ether, transferring the extract to a clean dry test tube and comparing it against a set of previously prepared standards. As it is in the choice of suitable standards and their carotin value that the utility of the method depends, it is necessary to deal with this in some detail.

The Selection of Standard Solutions.—

When this investigation was commenced Rabinowitch's method was followed, utilizing as standards petroleum ether solutions of oleic acid, but these were found to be unsatisfactory. Oleic acid is an oily liquid at ordinary temperatures, and when chemically pure is colorless. Being an unsaturated compound it readily oxidizes on exposure to air, and the greater the amount of impurity present the greater is the color which it possesses. Rabinowitch himself admits that his standard solutions deteriorate and should be renewed weekly; for a permanent standard he recommends the potassium dichromate solution adopted by Connor.[13] Another objection to the use of oleic acid is the difficulty of obtaining uniformity of coloration when the acid is obtained from different sources. We compared a number of samples of different grades of oleic acid which were in stock, in each case making up a 50 per cent solution in petroleum ether. Taking the purest sample as equivalent to 5 units of pigment (*vide* Rabinowitch), four samples had values of 5, 11, 20, and 30 units respectively, whilst two very impure samples were so strongly colored as to be noncomparable. It was accordingly decided to use potassium dichromate as the standard. A 0.2 per cent solution was made up and taken empirically as 100 units, and a series of test tubes (each of monax glass and 13 mm. bore) prepared, containing the dichromate solution suitably diluted to represent 1 to 100 units. These test tubes, sealed and kept out of contact with light, contained the series of standards. It still remains to determine their carotin equivalent. No pure carotin being available at that time, a carrot was extracted with petroleum ether, and without further manipulation the strongly colored extract was diluted with petroleum ether until it gave almost exact color comparison with the 0.2 per cent dichromate solution, both solutions being compared in test tubes of 13 mm. bore, using a simple color comparator. This extract was then progressively diluted with the solvent until a series of dilutions were obtained similar to those of the dichromate. On comparing these diluted carotin solutions with the corresponding dichromate solutions, it was observed that, as noted by Connor, progressive dilutions of the two solutions did not give corresponding values. The values were therefore plotted against one another and were seen to lie approximately on two straight lines which intersected at a point equivalent to 47.5 dichromate units. To verify this unexpected finding, on two other occasions petroleum ether extracts of carrots were made, progressively diluted, compared with the dichromate standards, and the results plotted. Although the carotin content of these extracts varied, in each case a graph similar to the first one was obtained with the point of intersection having the same dichromate value, 47.5 units. Further, the results from all three experiments when plotted together, could be represented by one pair of intersecting straight lines. In order to relate this to actual carotin content, a sample of crystalline carotin was prepared from carrots, and recrystallized. It had a melting point of 167°-168° C. (uncorr.). A solution containing 4.0 mg. of the crystals in 100 c.c. was found to give color comparison with 111 dichromate units (100 units being represented by a 0.2 per cent solution). Fig. 1 therefore represents the values

expressed in mg. carotin per 100 c.c. of a 0.2 per cent solution of potassium dichromate and its progressive dilutions.

The reason for the sudden change in the angle of inclination of the graph is unknown. It is not a property of the dichromate solution; this has been tested and found to give a straight line when the color intensity is plotted against the concentration throughout the whole range. The explanation may lie in the fact that the petroleum ether used (Merck's c.p., B.P. 30°-80° C.) was a mixture and not a true chemical compound, but in any case it is only of theoretical interest, since our experience has shown that even most pronounced cases of carotinemia have values which lie in the lower segment of the graph. This part of the graph can be represented by the equation:

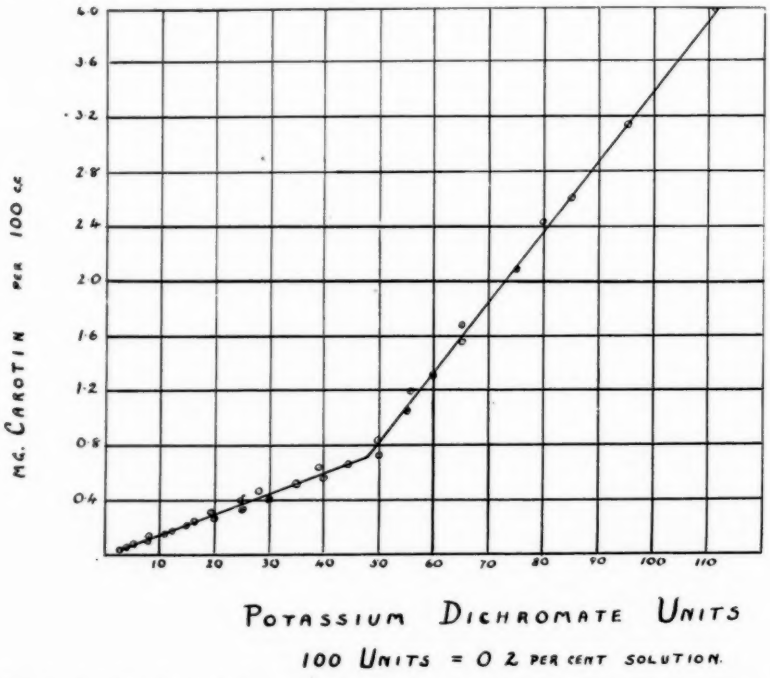


Fig. 1.—Graph showing the carotin values of potassium dichromate units at a depth of 13 mm.

$x = 0.375\ y$ (where y = number of dichromate units and x = number of carotin units), and as each carotin unit represents 0.04 mg. of carotin in 100 c.c. of petroleum ether, the equation can be further simplified and written thus: $X = 0.015\ y$ (where X = mg. carotin per 100 c.c.).

As previously mentioned, Willstätter and Stoll's results showed that a dichromate solution gave varying values when compared against the same carotin solution at different depths of field. It is not therefore possible to contrast their results with varying concentrations of dichromate and carotin solutions at the same depth of field, such as is now represented graphically. For the same reason Connor's tabulated data of the carotin value of different strengths of dichromate solution at a depth of field of 30 mm., are not comparable with our results which were obtained with a depth of field of 13 mm.

Details of the method of estimation are as follows: Two c.c. of serum are pipetted into a graduated centrifuge tube, and to this is added (in small portions) 4 gm. of plaster of Paris, the serum being stirred after each addition by means of a thin glass rod. When all has been added the contents of the tube should have the consistency of a thick paste. Two c.c. of 95 per cent alcohol are then added, and after again mixing thoroughly the glass rod is withdrawn, and 2 c.c. of petroleum ether pipetted in. The tube is immediately stoppered with a close fitting cork stopper (rubber is attacked by petroleum ether), shaken either by hand or (preferably) mechanically for ten minutes and then centrifuged. The contents will be found to have separated into 3 layers, of which the top contains all the carotin pigment. The top layer is measured and transferred to a clean dry test tube of 13 mm. bore, which is tightly stoppered, and the colored liquid compared against the dichromate standards until a match is obtained. The value in dichromate units being thus obtained, from the equation the equivalent value in terms of carotin is calculated. As this is expressed in mg. per 100 c.c., a correction has to be applied to compensate for the solution of a fraction of the petroleum ether in the alcohol layer. This is allowed for by multiplying by a factor, obtained by dividing the measured amount of the petroleum ether layer, by the volume of petroleum ether taken. After some practice we found that the petroleum ether layer could be taken as 1.8 c.c., giving a factor of 0.9. In Table I is shown a summary of results obtained with this procedure.

TABLE I

NO. OF CASES	DESCRIPTION	MG. CAROTIN PER 100 C.C. SERUM	
		MEAN	EXTREMES
18	Normal	0.063	0.024 – 0.108
14	Diabetic	0.213	0.072 – 0.379
8	Pathologic but nondiabetic	0.055	0.032 – 0.092

The Influence of the Serum Carotin upon the Icterus Index.—The icterus index introduced by Meulengracht[14] as a measure of the degree of bile pigmentation of serum, consists simply in matching the color of the serum against a standard solution of potassium dichromate, and is largely used as a routine test in cases of jaundice. In such cases it is assumed that the amount of carotinoid pigment present is not markedly increased, and that consequently the index gives a fairly reliable indication of the amount of bilirubin present in excess of normal. As amended by Maue[15] the procedure consists in comparing in the colorimeter a suitably diluted serum against the standard, the icterus index being calculated from the formula:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{dilution.}$$

It follows that when the standard and unknown readings are equal the icterus index is equal to the dilution. The standard used is a 0.01 per cent solution of potassium dichromate, so that if a 0.2 per cent solution is represented by 100 units, then the more dilute solution represents 5 units which is equivalent to 0.075 mg. carotin per 100 c.c. The highest value which we obtained for

nondiabetic serum was 0.108 mg. carotin per 100 c.c., so that the color due to carotin in this serum could be represented by an icterus index of 1.4. The normal range for the icterus index according to Bernheim[16] is 4 to 6, and these limits are generally accepted, consequently the color due to carotin in the sera of nondiabetic individuals apparently can have but little influence upon the icterus index. With cases of diabetes, however, the carotin content increases considerably. Of the 14 cases reported in Table I the highest value obtained was 0.379 mg., equivalent to about 25 dichromate units. This is five times the strength of the icterus index standard, and can therefore be represented by an index of 5. This superimposed upon the normal value brings the index within the zone of latent jaundice. On the other hand such a value would be accompanied by xanthosis and other symptoms which could be recognized clinically. It can be concluded therefore that except in cases of well-marked carotinemia such as sometimes accompany diabetes, the color of the serum due to carotin has very little effect upon the icterus index.

SUMMARY

Details are given of a modification, suitable for routine determinations, of Palmer's method for the estimation of serum carotin. It can be carried out on 2 c.c. of serum and the results expressed with reasonable accuracy in terms of mg. carotin per 100 c.c. serum.

It is concluded that, except in cases of marked xanthemia accompanying diabetes, the carotinoid pigmentation of the serum does not influence the icterus index to any appreciable extent.

We wish to express our indebtedness to Dr. C. Hunter, on whose suggestion this investigation was undertaken, and in addition, to Drs. F. J. Hart and N. W. Warner, for permission to investigate their cases and for their interest and cooperation. We also wish to thank Professor A. T. Cameron for his criticism and advice.

REFERENCES

1. Palmer, L. S.: Carotinoids and Related Pigments, New York, 316 pp., 1922.
2. Boeck, W. C., and Yater, W. M.: Xanthemia and Xanthesis (Carotinemia); Clinical Study, J. Lab. & Clin. Med. **14**: 1129, 1929.
3. Stannus, H. S.: Hyperlipochromia; Carotinaemia; Xanthosis Cutis, Internat. Clinics, Series 39 **1**: 146, 1929.
4. v. Euler, B., v. Euler, H., and Hellström, H.: A-Vitaminwirkungen der Lipochrome, Biochem. Ztschr. **203**: 370, 1928.
5. v. Euler, H., Karrer, P., and Rydbom, M.: Ueber die Beziehungen zwischen A Vitaminen ind Carotinoïden, Ber. chem. Ges. **62**: 2445, 1929.
6. Moore, T.: Vitamin A and Carotene. I. The Association of Vitamin A Activity With Carotene in the Carrot Root, Biochem. J. **23**: 803, 1929.
7. Moore, T.: Vitamin A and Carotene. VI. The Conversion of Carotene to Vitamin A *in vivo*, Biochem. J. **24**: 692, 1930.
8. Drummond, J. C., Ahmad, B., and Morton, R. A.: Further Observations on the Relation of Carotene to Vitamin A, J. Soc. Chem. Ind. **49**: 291 (T), 1930.
9. Willstätter, R., and Stoll, A.: Untersuchungen über Chlorophyll, Methoden und Ergebnisse, Berlin, 424 pp., 1913, quoted by Palmer.[1]
10. van den Bergh, A. A. H., and Snapper, J.: Die Farbstoffe des Blutserums, Deutsch. Arch. f. klin. Med. **110**: 540, 1913.
11. van den Bergh, A. A. H., Müller, P., and Broekmeyer, J.: Das lipochrome Pigment in Blutserum und Organen, Xanthosis, Hyperlipochromämie, Biochem. Ztschr. **108**: 279, 1920.
12. Rabinowitch, I. M.: Carotinaemia and Diabetes, Canad. M. A. J. **18**: 527, 1928; Carotinemia and Diabetes; Relationship Between Sugar, Cholesterol and Carotin Contents of Blood Plasma, Arch. Int. Med. **45**: 586, 1930.

13. Connor, C. L.: Studies on Lipochromes. III. The Quantitative Estimation of Carotin in Blood and Tissues, J. Biol. Chem. **77**: 619, 1928.
14. Meulengracht, E.: Die klinische Bedeutung der Untersuchung auf Gallenfarbstoff in Blutserum, Deutsche Arch. f. klin. Med. **132**: 285, 1920.
15. Maue, H. P.: Icterus Index of Blood Serum, Surg. Gynec. Obst. **34**: 752, 1922.
16. Bernheim, A. R.: Icterus Index (a Quantitative Estimation of Bilirubinemia); Aid in Diagnosis and Prognosis, J. A. M. A. **82**: 291, 1924.

THE EFFECT OF AMYL NITRITE UPON THE FINGER VOLUME*

By Carl A. Johnson,† M.D., Chicago, Ill.

THE prevalent opinion as to the action of amyl nitrite upon the peripheral vascular system is that of a generalized vaso dilatation, the effect being more pronounced in the face and neck region than elsewhere.

Accepting this view, we wished to use this drug as a control drug in a study of the factors modifying finger volume. Our results were rather striking and hence we felt justified in making this brief report.

PROCEDURE

Many plethysmographs have been described, but for the particular purpose at hand we used a specially constructed instrument illustrated in Fig. 1.

It consists of 1 c.c. pipette graduated to 0.01 c.c. fused to a one inch test tube. The test tube is cut off to any desired length and a glass stopcock fused to the side. The open end of the test tube is covered with a rubber dental dam with a hole sufficient to admit a finger snugly.

A drop of alcohol containing some pigment such as ink is allowed to run to the center of the pipette. The glass stopcock is kept open, one finger is put over the open end of the pipette, the finger to be tested is inserted into the plethysmograph and the individual takes a comfortable position. The glass stopcock is then closed.

With the average individual the deflection with each heart beat is approximately 0.01 to 0.02 c.c. (2 to 3 mm.) while with some cases of aortic regurgitation the deflection may be as great as 0.05 to 0.06 c.c. with each heart beat.

Time is allowed for an equilibrium to be reached within the tube for vapor and temperature changes will modify the readings. This usually takes about fifteen minutes. After a suitable control period, amyl nitrite was given by inhalation and readings taken. Normal healthy individuals were used throughout the work and in the sitting posture.

RESULTS

The results are given in Table I. From this it is seen that for some individuals the finger volume decreases immediately following amyl nitrite amounting to about 0.20 or 0.25 c.c. It will be noted that in one case (M)

*From the Department of Medicine of Northwestern University and St. Luke's Hospital, Chicago, Illinois.
Received for publication, April 7, 1931.
†Ciba Fellow in Medicine.

TABLE I

THIS TABLE ILLUSTRATES THE EFFECT OF AMYL NITRITE BY INHALATION UPON FIVE NORMAL INDIVIDUALS. THE READINGS ARE IN 0.01 C.C.

3/11/31 JOHNSON			3/14/31 FENN			3/14/31 BISSELL			3/14/31 YOUNG			3/16/31 MILLER		
TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE
3:12	0.4		11:45	0.3		12:57	0.26	92	1:34	0.32	66	10:15	0.3	
3:17	0.39		11:47	0.3	80	12:59	0.26	88	1:36	0.4		10:17	0.28	
3:20	0.41		11:49	0.2		1:00	0.24		1:38	0.49	66	10:18	0.3	
Amyl nitrite			11:50	0.19	76	1:01	0.23		1:39	0.45		10:19	0.31	
3:20½	0.19		11:51	0.23		Amyl nitrite			Amyl nitrite			10:20	0.3	
3:21	0.21		Amyl nitrite			1:02	0.43	136	1:40	0.34		Amyl nitrite		
3:22	0.24		11:51½	0.01		1:03	0.29	84	1:41	0.55		10:20½	0.09	
3:23	0.28		11:53	0.00		1:04	0.14		1:43	0.43	66	10:21	0.11	
3:24	0.30		11:54	0.03		1:05	0.13		1:44	0.43		10:22	0.12	
3:25	0.33		11:55	0.04		1:06	0.13		1:45	0.49		Fainted		
3:26	0.37		11:56	0.2		1:07	0.14		1:46	0.48				
			11:58	0.22										
			11:59	0.32										
			12:01	0.25										
Coughed so			12:02	0.35	76									
experiment			12:03	0.41										
discontinued			12:04	0.5										

the patient fainted. A decreased pulse rate was sometimes associated with the decreased finger volume. Following this the rate and finger volume usually increased above the control value. In one case the total change was 0.53 c.c. (Table I, Dr. F.)

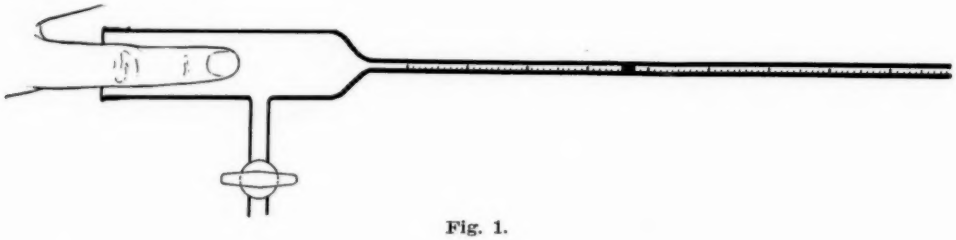


Fig. 1.

In contrast to the above series of events, some individuals show the reverse; i.e., primary increase in finger volume with a subsequent decrease of finger volume below the control value. (Table I, Dr. B.)

DISCUSSION

We have assumed without direct proof that sudden changes in the finger volume are due, in most part, to changes in the amount of blood in the finger vessels. We realize that slower changes in the readings may in part be due to temperature changes in the finger associated with changes in blood supply.

On this assumption we feel that the decreased finger volume in some individuals following amyl nitrite is due to the overwhelming splanchnic dilatation, decreased heart rate and amplitude with consequent fall in blood pressure. This causes a drainage of peripheral blood into the splanchnic region in spite of a possible definite relaxation of the vessels of the finger in question. In other words, blood pressure is insufficient to distend the vessels.

SUMMARY AND CONCLUSIONS

The author has attempted to show by means of a special finger plethysmograph that in some individuals a primary decreased finger volume occurs following amyl nitrite and attributes this finding to the drainage of peripheral blood into the splanchnic region. He is aware of the possibility that definite relaxation of the finger vessels may occur, but the blood pressure is insufficient to distend them.

THE VALUE AND LIMITATIONS OF THE ASCHHEIM-ZONDEK PREGNANCY TEST*

By Robert T. Frank, M.D., Morris A. Goldberger, M.D., and Gertrude Felshin, M.D., New York City, N. Y.

NO SATISFACTORY test for the recognition of early pregnancy was available until the Aschheim-Zondek test appeared in 1928.[1] This reaction is based upon an increase of the anterior pituitary lobe maturity factor in the urine of the pregnant woman. The discoverers of the test have been able to recognize pregnancy as early as three to four days after the expected period failed to occur.

A number of articles confirming the reports of Aschheim and Zondek have been published since 1928.[2, 3] None of them have contained modifications which have proved of value.

The Aschheim-Zondek test, in the hands of most investigators and clinicians who have reported on it, shows an accuracy of 95 to 98 per cent which, for a biologic test, is extremely satisfactory. Brouha,[4] however, obtained a positive reaction in only 60 per cent and Mazer and Hoffman[5] in 75 per cent.

Our own experience, covering some 350 tests, has proved satisfactory. Certain technical details, sources of error or difficulties, as well as comparisons with other methods of diagnosis, appear worthy of record.

The Aschheim-Zondek test was performed on the urines of 305 patients. Of these, 27 cases must be discarded because of the death of all the mice, in a few instances also on repetition. These were all among the first tests performed. The test should be repeated if only one mouse survives and its ovaries are found negative. By using only catheterized urine, or where this could not be obtained, by first passing the urine through a Berkfeld filter, the mortality of the mice has been reduced to a negligible figure.

At various times attempts at varying the technic were essayed. One of the recommended methods was the use of but two mice, each receiving 0.5 c.c. of urine for 6 doses. Trial showed that the results were less reliable.

Our standard technic differs in only one respect from that of the originators of the test, namely in the employment of four female immature mice

*From the Gynecological Service and Division of Laboratories of the Mount Sinai Hospital
Received for publication, May 7, 1931.

instead of five. The mice are injected subcutaneously, respectively with 0.2 c.c., 0.25 c.c., 0.35 c.c., and 0.4 c.c. at each injection, which are spaced two on the first, three on the second, and one on the third day. On the fourth day (100 hours after the first injection) the mice are killed with illuminating gas and at once autopsied.

Inspection of the ovaries in positive cases shows one or more minute purplish black spots in the ovaries (anterior pituitary reaction II; APR II) or less characteristic grayish yellow elevations (APR III) resulting from the luteinization of unruptured follicles. APR III can be confirmed as recommended by Aschheim and Zondek, by covering the ovaries with a drop of glycerin and flattening them between a glass slide and heavy cover glass. The luteinized follicles or corpora lutea can then be distinguished by their more opaque and granular appearance, from primordial or maturing graafian follicles, if at once examined under the low power of the microscope. This difference in opacity disappears within fifteen minutes as the glycerin "clears" the tissues. In rare instances, if this crude but rapid method proves indecisive, serial sections of the ovaries must be cut.

Opening of the vagina, as well as enlargement of the uterus and maturation of follicles (APR I) although due to the presence of hypophyseal hormone, signifies absence of pregnancy.

In the 321 tests which were completed, 10 errors, or 3.1 per cent, occurred, all in the direction of negative results in the presence of pregnancy as demonstrated by operation, follow up, or repetition of the test at a later time. In no case was the test positive in the absence of pregnancy. We now regularly crush and examine all macroscopically negative ovaries as, in a not inconsiderable number of instances, corpora lutea indistinguishable to the naked eye or to the loupe are thus recognized.

In several instances, especially in the first weeks of pregnancy, a negative reaction was noted, followed in a few weeks, on repetition, by a positive result.

Death of fetus did not affect the result as long as the products of conception were retained in utero even for weeks or months. This is in sharp contrast with the blood female sex hormone test[6] which, as a rule, at once becomes negative. The sole exception to these observations was a case of "missed abortion" with detachment and retention of the ovum, in which the Aschheim-Zondek test was also negative.

Of eleven ectopic gestations, eight gave a positive reaction. One negative reaction was noted in a urine obtained two days after operation, another was accounted for by the complete degeneration of the ectopic ovum. Consequently but one negative result can be ascribed directly to failure of the reaction.

Mazer and Hoffman[7] described a urine reaction in which 10 c.c. of suspect urine is injected in 5 divided doses into mature castrates. In only 75 per cent of pregnancies was a positive reaction obtained. In 4 per cent of nonpregnant urines their test proved positive. This high percentage of error in the presence of pregnancy may be accounted for by the fact that our studies[8] have shown that during the first eight weeks of pregnancy a nega-

tive phase in the production of female sex hormone takes place. The false positives, on the other hand, are due to excess excretion of female sex hormone noted by us[9] and others[10] in amenorrhea and in various disturbances of the cycle at the approach of the menopause.

The Siddall test,[11] which is based on the combined contents of female sex hormone and anterior pituitary hormone in the blood serum of pregnant women with the consequent increase in weight of the genital tract of the injected immature mice, gave 17 per cent of false positives in Mazer and Hoffman's hands. It should, therefore, not be employed.

Aschheim and Zondek[1] in 1928 observed that 1.0 to 0.5 c.c. of blood serum of pregnant women gave a positive reaction (APR II or III). Fluhmann[12] noted the same. We have found serum reaction (using 1.5 c.c.; 1.0 c.c., and 0.5 c.c., totals, divided into 6 injections) almost as reliable as the urine test.

In one case of hydatid mole the urine test was positive, as were the blood and urine female sex hormone tests.

In two cases of chorionepithelioma in the male, the Aschheim-Zondek test was positive (one primary chorionepithelioma of testis with retroperitoneal metastases; the second multiple chorionepithelioma of the lung, primary site undetermined). These cases will be reported elsewhere in more detail.

TABLE I

CLINICAL DIAGNOSIS	NO. OF CASES	TESTS		WRONG	REMARKS
		+	0		
Pregnancy before eighth week	94	72	35*	4**	*Two repeating became +. **Two negatives were pregnant.
Pregnancy after eighth week	13	11	2	2*	*One of these was + at 6 weeks but 0 at 4½ months.
Hydatid	2	1	2*	0	*Negative tests performed 6 weeks after emptying uterus.
Ectopic gestation	11	8	3*	1	*One negative test from urine 2 days after operation. One old tubal abortion, involuted.
Pregnancy vs. fibroids	9	5	4	0	
Dead vs. live fetus	9	6	8	2	Two positives with dead fetus. Two negatives with live fetus.
X-ray effect on pregnancy	10	10	14	–	Does not show death of fetus until weeks have passed.
Amenorrhea vs. pregnancy	80	0	85	0	Fully reliable.
Metrorrhagia vs. abortion	13	7	9	1*	One became negative on repeat. *One became positive on repeat.
Chorionepithelioma in male	2	2	0	0	
Follicle fluid	6	2*	4	0	*This was in pregnant patients.
Pituitary disease	16	0	18	0	
Miscellaneous	13	1	12	0	The one positive was pregnant, the others not.
All mice died	27	–	–	–	30 tests made; in 3, mice died on repeat.
Totals	305	125	196	10	3.1 per cent error.
		321			
(All mice died)		30			
Total Tests		351			

SUMMARY

1. The Aschheim-Zondek test, if the original technic is followed, is satisfactory.

2. Failure of positive result in pregnancy occurred in 3.1 per cent.

3. If the ovaries are negative macroscopically, it is advisable to examine the fresh, crushed ovaries under the microscope.

4. No positive reaction, in the absence of pregnancy, was noted in our series.

5. Death of fetus may not influence the reaction for weeks. The female sex hormone blood reaction is more delicate under these circumstances.

6. The Aschheim-Zondek reaction is also reliable in the diagnosis of ectopic gestation.

7. Chorionepithelioma in the male produces a positive reaction.

8. The blood serum gives an accurate Aschheim-Zondek test. Further report on this will appear later.

Our experience with the intravenous Friedman[13] rabbit pregnancy test, which is said to give a reaction in twenty-eight to thirty-six hours, is insufficient to permit of comment or judgment.

REFERENCES

1. Aschheim, S., and Zondek, B.: Schwangerschaftsdiagnose aus dem Harn (Durch Hormonnachweis), Klin. Wchnschr. **7:** 8, 1928.
2. Liese, G., and Auer, E. S.: The Biologic Diagnosis of Early Pregnancy by the Aschheim-Zondek Test, Am. J. Obst. & Gynec. **20:** 667, 1930.
3. Mack, H. C., and Catherwood, A. E.: The Aschheim-Zondek Reaction in Hydatidiform Mole and Malignant Chorionepithelioma, Am. J. Obst. & Gynec. **20:** 670, 1930.
Mathieu, A.: Pregnancy Tests, Med. Sentinel, Nov., 1929.
Erhhardt, K.: Beitrag zur Hypophysenvorderlappen: Reaktion inter Besonderer Beruchtsictigung der Aschheim-Zondekschen Schwangerschaftreaktion, Klin. Wchnschr. **8:** 2044, 1929.
4. Brouha, L., Hinglais, H., and Simonnet, H.: Biologic Diagnosis of Pregnancy, Paris Méd. **1:** 221-236, 1930.
5. Mazer, C., and Hoffman, J.: The Three Hormone Tests for Early Pregnancy, J. A. M. A. **96:** 19, 1931.
6. Frank, R. T., and Goldberger, M. A.: Clinical Data Obtained With the Female Sex Hormone Blood Test, J. A. M. A. **90:** 106, 1928.
7. Mazer, C., and Hoffman, J.: The Diagnosis of Early Pregnancy Through the Detection of Female Sex Hormone in the Urine, Am. J. Obst. & Gynec. **17:** 186, 1929.
8. Frank, R. T.: The Female Sex Hormone, p. 194, Charles C. Thomas, Springfield, Ill., 1929.
9. Frank, R. T., and Goldberger, M. A.: The Female Sex Hormone. XI. Utilization of the Hormone in the Normal Woman: Effect of Abnormal Kidney Permeability in the Production of Amenorrhea and Sterility, J. A. M. A. **94:** 1197, 1930.
10. Zondek, B.: Die Hormone des Ovariums und des Hypophysenvorderlappens, p. 239, Julius Springer, Berlin, 1931.
11. Siddall, A. C.: The Hormone Test for Pregnancy, J. A. M. A. **91:** 779, 1928.
12. Fluhmann, C. F.: Anterior Pituitary Hormone in the Blood During Pregnancy, J. A. M. A. **92:** 1744, 1929.
13. Friedman, M. H.: Mechanism of Ovulation in the Rabbit. II. Ovulation produced by the injection of urine from pregnant women, Am. J. Physiol. **90:** 617, 1929.

THE CLINICAL INCIDENCE OF TRYPTOPHANURIA*

By Arthur T. Brice, Jr., B.A., Palo Alto, Calif.

IN THE presence of concentrated sulphuric acid the aldehydes react with the tryptophane groups of any proteins which contain such groups, producing the characteristic lilac or blue color of the Adamkiewicz reaction. B. S. Walker and F. H. Sleeper[1] have shown that the Boltz technic is a specific test for tryptophane based on this reaction as a result of the aldehydes present as impurities in all brands of acetic anhydride. The Boltz test has been extensively employed in the analysis of cerebrospinal fluids, and in November, 1930, I reported its application to urine[2] together with the results of a brief series of normals indicating that tryptophane is not eliminated in quantities detectable by Boltz's test in the urine of the normal male adult in health and on an average diet, and a series of 525 tests on specimens from general surgical and medical cases. This series has now been extended to include over 600 tests from cases of nervous and mental diseases, accumulated during recent months.

During the course of the application of the test to date the following observations having a technical bearing have been made. Positive specimens left standing at room temperature overnight without a preservative showed in the morning considerable growth of bacteria and were negative by the test. The same specimens preserved with a few drops of chloroform, ether, or toluol remained positive. Positive specimens kept at room temperature in stoppered flasks have been preserved by the use of toluol for as long as a month. Mercuric chloride solutions precipitate tryptophane from urine and preserve it against bacterial decomposition. A very distinct improvement in the technic of the Boltz test when employed in uranalysis has been found to consist in cooling the tube containing the test solutions, either in a large beaker of water or under the tap, during the addition of the sulphuric acid. This keeps down to a minimum the production of interfering yellow and brown colors, and renders the test much simpler to read with certainty.

Twenty specimens from general surgical and medical cases have been observed and recorded giving positive Boltz tests in the presence of acetone and diacetic acid. Seven specimens from five different cases of diabetes mellitus have been observed and recorded positive for tryptophane by Boltz test in the presence of sugar. The presence of acetone, diacetic acid, and sugar, even in considerable amounts, therefore, does not necessarily mask a positive Boltz test. The removal of ammonia by permutit, or of phosphates and carbonates by barium chloride and hydroxide, or of carbohydrates by copper sulphate and calcium hydroxide, from the average specimen of urine does not appreciably simplify the reading of the Boltz test or increase its sensitiveness through any clarification of interfering colors. The exact sensitiveness of

*From the U. S. Veterans Hospital.
Received for publication, April 28, 1931.

the test up to the present time has not been determined. It is believed to be amply sensitive to demonstrate a clear-cut easily readable reaction with pathologic positives.

Tryptophane was found to be present in 33 per cent of 525 specimens examined from general surgical and medical cases. Of 313 of these specimens which were positive for albumin 91 or 29 per cent were also positive for tryptophane. The highest incidence of tryptophane in combination with albumin of any group of specimens from similar cases was shown in the group of 25 specimens from nine different cases of degenerative diseases of the kidneys, eighteen of which or 72 per cent were also positive for tryptophane. Two hundred and twenty-two specimens positive for albumin by the usual routine tests were negative for tryptophane by Boltz test, indicating the existence of an albuminuria in which the protein molecule eliminated does not contain a tryptophane group. One hundred and sixty-nine specimens in this series were found positive for tryptophane by Boltz test, of which number 78 or 46 per cent were albumin free. This indicates the existence of a tryptophanuria as a clinical entity distinct from albuminuria.

The highest incidence of free tryptophane not in combination with albumin was found in the small group of five cases of nervous disorders such as menopause, neuritis, neurasthenia, hysteria, neuroretinitis, and seven cases of surgical shock such as amputation, gunshot and other wounds, fracture, dislocation, and secondary hemorrhage. The figures while meager led me none the less to the conclusion that tryptophanuria as a distinct clinical entity might most likely be found in the group of nervous and mental disorders. This conclusion has been partially verified by the examination of over 600 specimens from 101 cases of mental disease as follows: miscellaneous diagnoses 7; general paresis 7; manic depressive psychosis 7; dementia precox 80. The average incidence of tryptophane positive specimens was slightly less than in general surgery and medicine, being but 21 per cent, this finding, however, representing almost entirely free tryptophane not in combination with albumin. Of the 101 cases examined by the test eight in the dementia precox group have been found who consistently run a tryptophanuria with no concurrent albuminuria.

The evidence is rather strongly indicative that tryptophanuria in nervous and mental disease is most likely to occur during periods of hyperactivity. This conclusion was reached by a consideration of the findings in individual cases and is supported by the following figures: Of 350 specimens taken at random from the four halls of one ward of the United States Veterans Bureau Hospital No. 24 at Palo Alto, California, 50 specimens or slightly over 14 per cent were found positive, while of 258 specimens taken in the tub room of the same ward 79 or about 31 per cent were positive. The findings with reference to the effect of tubs and packs indicate that this treatment is more likely to increase a tryptophanuria than to diminish it.

REFERENCES

1. Walker, B. S., and Sleeper, F. H.: Tryptophane Reactions in Spinal Fluid, J. Lab. & Clin. Med. 12: 1048, 1927.
2. Brice, A. T., Jr.: The Boltz Test in Urinalysis, Arch. Int. Med. 46: 778, 1930.

COMPARISON OF THE HUDDLESON SLIDE TEST WITH A MACROSCOPIC TUBE TEST IN UNDULANT FEVER*

By Henry Welch, Ph.D., and Friend Lee Mickle, M.S., Hartford, Conn.

THE literature on the relative merits of the agglutination test for undulant fever indicates that the Huddleson test is as specific and as sensitive as the macroscopic tube test. Huddleson and Abell[1] have tested thousands of serums with the rapid (Huddleson) method and find it as accurate and specific as any other method of detecting the presence of specific antibodies for Brucella abortus. Damon,[2] Palmer and Baker[3] and Lienhardt and Kitselman[4] all report the Huddleson test as accurate as the macroscopic tube test.

These Laboratories have been reporting on the presence of agglutinins for the Brucella group in blood sera since November 19, 1926. This work up to July, 1930, has been published[5, 6, 7, 8, 9] and with investigational studies included some 20,500 sera examinations. The sera on reaching the Laboratories were sent to the Connecticut (Storrs) Agricultural Experiment Station where the macroscopic tube test was made first by Dr. J. G. McAlpine and later by Dr. W. N. Plastridge, the results reported to these Laboratories and from here subsequently to the physician. The time required caused considerable delay before the physician received his report. It was felt that by the use of the Huddleson technic, which is a considerably shorter method for diagnosing undulant fever, the test could be made in our own Laboratories at a great saving in time. Accordingly, the Huddleson rapid method has been run routinely on all specimens sent to the Laboratories for diagnosis of undulant fever for the past five months. The results obtained have been compared with the results of the macroscopic tube test made in the Storrs laboratory.

METHODS

Huddleson Rapid Method.—The apparatus used in the Huddleson test has been described[1] and is easily constructed in the laboratory. For our investigation a box of 16.5 × 11 × 6 inches containing two 50-watt electric light bulbs was used. (Note Fig. 1.) The top of the box was partially covered with half-inch wood five inches wide to protect the eyes of the technician reading the tests. The light bulbs were attached to either end of the box so as to be under the five-inch strip of wood. A piece of plate glass lined off in one and one-half inch squares covered the rest of the top of the box.

The Huddleson antigen is standardized for use with undiluted sera in the following amounts 0.08 c.c., 0.04 c.c., 0.02 c.c., 0.01 c.c. and 0.004 c.c., corresponding to dilutions of 1:25, 1:50, 1:100, 1:200 and 1:500 in the tube test. For comparative purposes agglutinations were made out to the 1:3000 dilution. It was found possible to do this by a preliminary comparison of the

*From Bureau of Laboratories, Connecticut State Department of Health.
Received for publication, May 1, 1931.

Huddleson antigen in a tube test (diluted) and in a regular slide test (undiluted).

Each specimen of blood on reaching the Laboratories was centrifuged and the clear sera decanted. A portion of this sera was sent to the laboratory at Storrs for the macroscopic tube test, and the remaining portion distributed in varying amounts to produce dilutions 1:25 through 1:3000 (note Table II) on the plate glass. One drop of antigen—Huddleson from a standardized dropper was then added to each dilution. The antigen and sera were mixed with clean toothpicks and the plate glass rotated for two to three minutes. The lights were then turned on inside the box and the degree of clumping in the different dilutions estimated. Occasionally in questionable positives we found it possible to obtain more accurate readings by placing the plate glass on a black background and reading by direct light with a dissecting microscope.

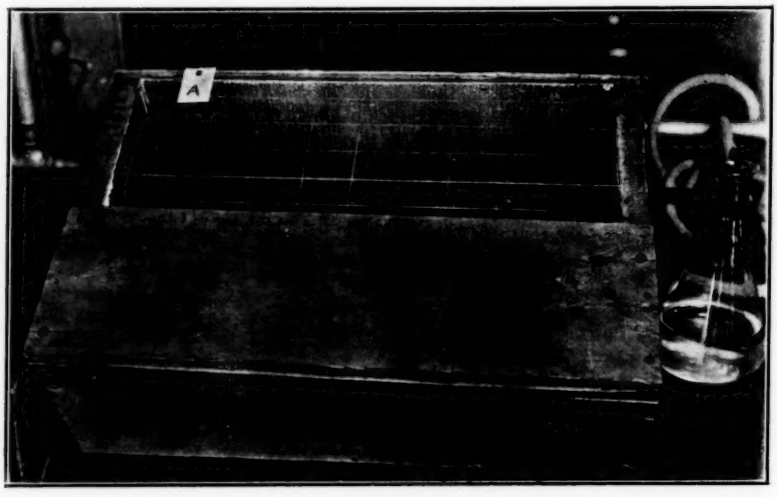


Fig. 1.—Apparatus used for Huddleson test.

Macroscopic Tube Test.—The antigen employed in the tube test was made from four strains of Brucella abortus, coming from different sections of the country, whose agglutinability had been carefully checked. These strains were grown for twenty-four hours on 1 per cent glycerin Fairchild's agar, and from these transplants were made upon plain Fairchild's agar and incubated forty-eight hours at 37° C. The growth was harvested with a small amount of 0.4 per cent carbolized saline solution and diluted for the final test to a nephelometer 1 on the McFarland[10] Scale. This diluted antigen was distributed in small test tubes in 2 c.c. amounts and the serum added to make final dilutions of 1:25 to 1:3000 as shown in Table II. After the addition of antigen the tubes were corked and shaken, incubated for forty-eight hours at 37° C., and one reading was made on the removal from the incubator and a second after twenty-four hours at room temperature.

RESULTS

A total of 156 sera were examined. Of these sera 20 were positive from a clinical and laboratory standpoint by both tests, there were 122 negative with both tests, 10 questionable reactions with the tube test and two with the Huddleson rapid test. These results are summarized in Table I.

TABLE I

RESULTS OF HUDDLESON AND MACROSCOPIC TUBE TESTS ON 156 SERA BETWEEN AUGUST 12, 1930 AND DECEMBER 31, 1930

	WITH HUDDLESON	WITH TUBE TEST	WITH BOTH
Positive tests	20	20	20
Negative tests	130	122	122
Questionables*	2	10	10†
Repeated positives	4	4	4
Total tests	156	156	156

*Clinically negative.
†Includes ten questionable tube tests two of which also were questionable with Huddleson.

An examination of this table shows that there is no difference in sensitivity with either test in the positive cases of undulant fever. However, there were eight more questionable reactions with the tube test than with the Huddleson which indicates a lesser degree of specificity of this tube test. The titers in this series of eight questionable reactions in the tube test varied from 2+ agglutinations in the 1:75 dilution to 2+ agglutination in the 1:25. In no cases were definite agglutinations noted in the 1:100 dilution.

In order to show the relationship of the Huddleson rapid test and the macroscopic tube test more accurately the 20 positive tests with their titers are given in Table II. In each of these cases a clinical diagnosis of undulant fever was made.

By examination of Table II it will be seen that the Huddleson test gave higher titers than the macroscopic tube test in eleven of the 20 tests. Two of the macroscopic tube tests were slightly higher than those of the Huddleson, whereas seven tests agreed in titer. It is obviously not our intention to place too much emphasis on differences in titers by these two tests. Differences in technic and technicians will probably account for it, but it is interesting that in using such a series as this that the Huddleson rapid test did seem to show titers somewhat higher than the macroscopic tube test.

DISCUSSION

According to the Illinois Undulant Fever Commission,[11] a positive reaction in a titer of 1:100 is considered diagnostic for undulant fever. In the series of 20 positive cases reported in this paper only in one case (note sera No. 69412) was a tube test titer as low as 1:100, and in this case the Huddleson rapid test showed a much higher titer (1:1500). In our experience the titers of sera from cases of undulant fever are rarely as low as the 1:100 dilution. In all 20 cases the Huddleson test showed agglutination in the 1:1000 dilution or better. Clinical cases with low titers reported by workers elsewhere have not been encountered in Connecticut.

TABLE II

DEGREE OF AGGLUTINATION WITH HUDDLESON RAPID TEST AND MACROSCOPIC TUBE TEST ON TWENTY CLINICAL CASES OF UNDULANT FEVER

DILUTION OF SERA		25	50	75	100	150	200	300	500	750	1000	1500	3000
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
57522	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	3+	3+	3+	+
61207	M	4+	4+	4+	4+	4+		4+	4+	3+	2+	?	–
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
61923	M	3+	4+	4+	4+	4+		4+	4+	4+	4+	3+	2+
	H	3+	3+		3+		3+		3+	2+	2+	1+	–
69412	M	4+	4+	3+	2+	?		–	–	–	–	–	–
	H	4+	4+		4+		4+		3+	2+	1+	1+	–
69822	M	2+	4+	4+	4+	4+		2+	?	–	–	–	–
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
70677	M	?	3+	4+	4+	4+		4+	4+	4+	4+	3+	2+
	H	4+	4+		4+		4+		4+	4+	3+	2+	2+
73790	M	4+	4+	4+	4+	4+		4+	4+	2+	?	–	–
	H	4+	4+		4+		4+		3+	2+	1+	?	–
76511	M	4+	4+	4+	4+	4+		3+	?	–	–	–	–
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
76787	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	3+
78334	M	4+	4+	4+	4+	4+		4+	4+	4+	3+	2+	–
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
79025	M	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	2+
	H	4+	4+		4+		4+		4+	4+	4+	3+	2+
79469	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
79597	M	?	?	2+	4+	4+		4+	4+	3+	2+	?	–
	H	4+	4+		4+		4+		4+	4+	4+	2+	–
80158	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	2+	?
	H	4+	4+		4+		4+		4+	3+	3+	2+	–
80160	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	3+	2+
	H	4+	4+		4+		4+		4+	4+	4+	4+	3+
80957	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	2+
	H	2+	2+		4+		4+		4+	4+	4+	4+	4+
81728	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
81924	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	3+	2+	–
82280	M	?	2+	3+	3+	3+		3+	3+	2+	2+	–	–
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
521	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+

4+ Complete Agglutination.
3+ 75% Agglutination.
2+ 50% Agglutination.
+ 20% Agglutination.
– % Agglutination.
H = Huddleson macroscopic test.
M = Macroscopic tube test.

The questionable reactions noted were obtained in most instances from cases of typhoid fever or else from patients who had had typhoid vaccine within a year. Whether these reactions were cross agglutinations with typhoid or natural agglutinins for the Brucella group in the blood stream of the patient is difficult to say. The fact that the patients had typhoid fever or typhoid vaccine is at least suggestive. Gilbert and Coleman[12] report the presence of typhoid agglutinins in the blood stream of patients with febrile diseases who had never received typhoid vaccine or to their knowledge had typhoid fever. It may well be that the questionable reactions we obtained were of a similar nonspecific nature being induced by previous injections of typhoid vaccine or a previous infection with the typhoid bacillus.

Our results indicate further that the Huddleson rapid method is slightly more sensitive and specific than the tube test used for comparison. Whether these differences observed were due to the type of macroscopic tube test used or indicate a superiority of the Huddleson technic over that of the tube test cannot be stated definitely without further work with a variety of antigens and tube test technics. However, we do feel that for our routine examinations, considering the ease and rapidity of the test, the sensitivity and specificity of the antigen and the time saved in getting reports to our physicians, the Huddleson rapid method is far superior to that of the macroscopic tube test.

SUMMARY

A series of 156 specimens of blood tested for the presence of agglutinins for undulant fever with a macroscopic tube test and the Huddleson rapid slide method indicates that the latter test is slightly more specific and sensitive than the former. Titers as low as 1:100 dilution in positive cases occurred in one case only with the tube test and the Huddleson in all positive cases showed typical agglutination in the 1:1000 dilution or higher. The rapidity and ease with which the Huddleson method can be carried out makes it an excellent test for laboratories where the diagnosis of undulant fever is carried out routinely.

REFERENCES

1. Huddleson, I. F., and Abell, E.: Rapid Macroscopic Agglutination for the Serum Diagnosis of Bang's Abortion Disease, J. Infect. Dis. **42**: 242, 1928.
2. Damon, S. R.: A Comparison of the Rapid and Slow Agglutination Methods for the Diagnosis of Bang Abortion Disease in Cattle, J. A. V. M. A. **75**: 761, 1929.
3. Palmer, C. C., and Baker, H. R.: Correlation of Rapid and Slow Agglutination Tests for Bang's Abortion Disease of Cattle, J. A. V. M. A. **75**: 86, 1929.
4. Lienhardt, H. F., and Kitselman, C. H.: Correlation of the Rapid and the Long Agglutination Tests for Infectious Abortion of Cattle, J. A. V. M. A. **73**: 328, 1928.
5. McAlpine, J. G., and Mickle, F. L.: Bacterium Abortus Infection in Man. The Results of the Agglutination Test Applied to More Than 10,000 Human Sera, Am. J. Public Health **8**: 609, 1928.
6. Mickle, F. L.: Annual Report of Connecticut State Department of Health, p. 218, 1927.
7. Mickle, F. L.: Annual Report of Connecticut State Department of Health, p. 293, 1928.
8. Mickle, F. L.: Annual Report of Connecticut State Department of Health, p. 318, 1929.
9. Mickle, F. L.: Annual Report of Connecticut State Department of Health, 1930, p. 386.
10. McFarland: Nephelometer; An Instrument for Estimating the Number of Bacteria in Suspensions Used for Calculating the Opsonic Index and for Vaccines, J. A. M. A. **49**: 1176, 1907.
11. Educational Health Circ., Undulant Fever: Cause, Transmission and Control Methods, 1929 (Prepared by Illinois Undulant Fever Committee), 36, Illinois Dept. Public Health.
12. Gilbert, R., and Coleman, M. B.: Agglutination of Typhoid Bacilli in Serums of Patients Having Unrelated Infections, J. Infect. Dis. **46**: 311, 1930.

A PROPOSED STANDARD METHOD FOR THE EVALUATION OF FUNGICIDES*

By Adelia McCrea, Ph.D., Detroit, Mich.

TO THOSE who work with fungi, especially parasitic fungi, the problem of a safe and certain fungicide is of great importance. Much work has been done in the attempt to find or to develop effective agencies for the destruction of these pests of the field and laboratory.

In recent years, interest in this direction has rapidly increased due to widespread infection by fungi parasitic upon the human skin. These skin mycoses are encountered much more frequently at present than they were a decade ago, and doubtless the average physician has come to recognize them more readily as they become more common. This type of skin lesion bears many pseudonyms, e.g., golfer's itch, student's itch, athlete's foot, and, while not fatal, it is the cause of a tremendous amount of annoyance and lowered efficiency among those so afflicted.

In an attempt to compare the reports of various workers heretofore made on the germicidal properties of substances used for combating bacteria and fungi, it became evident (a) that no standard method has been adopted by workers in this field, and (b) that practically all of the tests heretofore made in vitro have been based on continued contact, i.e., have been *fungistatic* tests rather than *fungicidal* tests. To call a substance fungicidal unless it actually kills the organism is faulty terminology.

In connection with a study of two of the most common organisms encountered in mycoses of feet and hands, i.e., *Trichophyton interdigitale* Priestly and *Epidermophyton rubrum* Cast., the writer has developed a test method for fungicidal powers of agencies against these and other fungi, which it is hoped may be conveniently followed by other workers. Once such a method is established, it will be possible to compare very closely the results obtained by many workers, and should greatly decrease the amount of conflicting results in subsequent reports. In outlining the method below, an effort has been made to give all important details in full, but the writer will be glad to give any additional information which may be desired. The method follows in detail.

Medium Used.—Sabouraud's original formula for glucose agar medium was used except that American ingredients were necessary† since the French glucose is not obtainable. The formula is

Glucose (Merck)	4.0%
Peptone (Witte)	1.0%
Agar-agar	1.8%

*From the Research and Biological Laboratories, Parke, Davis and Company.
Received for publication, May 13, 1931.

†For purposes of isolation and identification of organisms, the French peptone Chassaing is used but this was not practical for the large amounts used in these tests.

This is made up with tap water, sterilized fractionally in flowing steam for one-half hour on three successive days, adjusted to neutral and stored in flasks or tubed at once as desired. This medium is used throughout the tests.

Reagents.—In reporting the value of any agency, it is most important that the *source should be given,* as well as the exact designation, e.g., "Auramine" is prepared by various manufacturers and may be listed as "Auramine 0," "Auramine 00," etc., the preparations often varying widely in their efficiency. Proper designation would be, e.g., "Auramine 0, Coleman and Bell," or whatever the case may be.

It is also to be realized that the question of penetration is significant in any study of lethal action, and that an ineffective substance, if properly buffered, may become of value, hence it is important that any and all such modifications be stated when data are given.

Physical Factors.—To reduce the variation due to these factors, the following conditions are adhered to: (a) incubation in the dark at a temperature of 22° to 28° C. (b) For agar slants, test tubes 16 × 140 mm. are used, with a butt of ⅜ to ½ inch. (c) Spore suspensions are made on the basis of 1 culture tube of growth taken up in 8 to 10 c.c. of sterile, distilled water. This inoculum is then strained through two layers of gauze (cheesecloth) to remove mycelial fragments and prevent "clumping" of conidia. It is then tubed and used within six hours after preparation in planting the test liquids.

Fungicidal Test.—This test involves exposing the heavy, strained, spore suspension for definite time periods to a freshly prepared solution of the desired strength of the reagent being tested. After each interval of exposure a portion of the material is transferred by loop to the surface of tubes of the standard medium, usually two loops per tube. The tubes are then incubated as above described, with the customary control cultures. The writer's practice is to add 0.5 c.c. of spore suspension to 2 c.c. of each test solution. At intervals of one minute, ten minutes, and one hour, portions of the mixture are taken by stirring with the loop and transferring to tubes of culture medium. Other quantities or time intervals could readily be adapted to this method, but, in that event, *they should always be definitely stated* for the benefit of later workers, and this holds also for the addition of one's choice of buffer or other modification of the method. Only by such cooperation may we eventually overcome the lack of harmony in the results of workers interested in this matter.

To give a concrete illustration, suppose, e.g., that a test is being made of the effect of gentian violet upon, say, a Trichophyton culture. The procedure would be as follows: 0.10 gm. of the dye is taken up in a few drops of alcohol and diluted with water to 25 c.c., giving a solution of 1 to 250. From this stock solution one readily prepares a dilution series of 1-250, 1-500, 1-1,000 and 1-5,000 which is sufficient range for a preliminary test. Each dilution is arranged in its respective tube, 2 c.c. per tube. To it is added 0.5 c.c. of spore suspension prepared as described above. Transplants are made to two tubes of the standard medium after one minute, ten minutes, and one hour, and placed in the dark cupboard. By "staggering" the intervals, the whole series of four dilutions and three time intervals can be completed in less than one

and one-fourth hours. Within the next forty-eight hours, often in twenty-four hours, growth will indicate which dilutions are ineffective, e.g., growth may be normal at 1-5,000 for all three time-exposures but 1-1,000 show growth only after one minute and ten minutes. This gives a middle ground from which to plan the final test. From the stock solution, kept in the refrigerator, dilutions are prepared of 1-600, 1-800 and 1-1,000 which are then tested exactly as before and give a final rating, if good judgment has been used in the dilution series chosen. Table I serves to illustrate the method.

FUNGICIDAL TEST

Organism: Trichophyton A.
Test substance: Gentian violet (C & B)

	DILUTION	ONE MINUTE	TEN MINUTES	ONE HOUR
Preliminary Series:				
	1-250	− −	− −	− −
	1-500	− −	− −	− −
	1-1,000	+ +	+ +	− −
	1-5,000	+ +	+ +	+ +
Final Series:				
	1-600	− −	− −	− −
	1-800	+ +	− −	− −
	1-1,000	+ +	+ +	− −

Interpretation: Gentian violet is fungicidal for this organism in one minute at 1-600, in ten minutes at 1-800, and in one hour at 1-1,000, but permits normal growth after an exposure of one hour at 1-5,000.

It is recognized (a) that the addition of the spore suspension to the test fluid increases the dilution, and (b) that in borderline cases additional time intervals may occasionally be desirable. However, the foregoing method gives a very good idea of the fungicidal activity of test substances and, from a pragmatic point of view, has proved most helpful in establishing comparative values.

A report is in progress of results obtained under the above method with a group of the aniline dyes and with various other materials, which it is expected will be published in the near future. This will show a compilation of the data obtained by this method in tests of approximately forty substances. Eleven organisms were used during these studies. When checked by application to the actual "ringworm" lesions in the guinea pig, the above "Fungicidal Test" was found to be of greater practical use than any other means of evaluation tried.

A RECORDING TYPE OF ARTIFICIAL PNEUMOTHORAX APPARATUS*

By Burgess Gordon, M.D., Philadelphia, Pa.

THE value of compression treatment in unilateral pulmonary tuberculosis (progressive) is recognized. Unfortunately the procedure is not widely employed. One of the reasons for this is the difficulty in operating the usual type of apparatus. The chief objections concern the water manometer used in determining the degree of thoracic pressure. The water is discharged easily on coughing or during the improper manipulation of pet cocks. This delays the

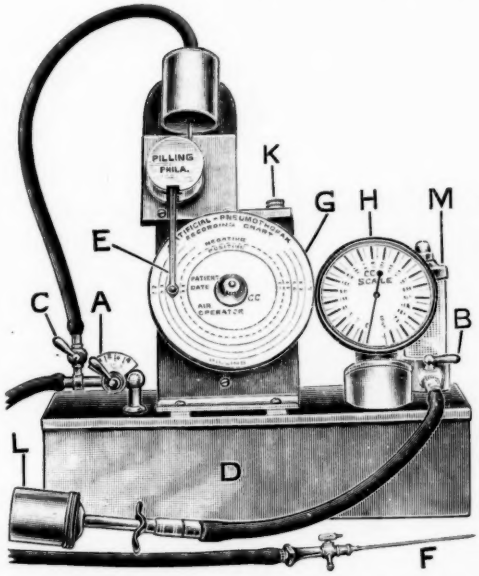


Fig. 1.—*E*, tracer; *G*, chart holder; *K*, release lever; *H*, pressure gauge; *M*, air filter; *L*, pump; *D*, air reservoir; *B*, pump pet cock; *C*, recording pet cock; *A*, air controlling pet cock.

operation and makes calculations difficult. The question of faulty procedure may be raised even with careful technic and efficient apparatus because a graphic record is not available. In addition the usual devices for controlling and estimating the amount of air passing into the chest are not entirely satisfactory.

An instrument has been devised to minimize these objections. (Fig. 1.) The following is the plan of construction: A copper chamber *D* especially plated on the inside to prevent corrosion replaces the glass jar (or jars) usually employed as an air reservoir. A compression pump *L* and filter *M* provide the mechanism for forcing dust-free air into the chamber. An aneroid

*From the Medical Service of Dr. Thomas McCrae and the Department for Diseases of the Chest, Jefferson Hospital, Philadelphia, Pa.

Received for publication, May 12, 1931.

pressure gauge *H* (recording up to 1050 c.c.) shows the amount of air in the reservoir. The variations in negative and positive pressure are recorded on a paper chart (Fig. 2) by means of a set-up consisting of pet cocks, bellows, connecting rod, gear box, tracing needle and kymograph. The chart holder *G* and the pressure gauge *H* are tilted at convenient angles so the operator may observe the variations without difficulty.

The instrument is operated as follows: The chart is placed in position on the kymograph, pet cock *A* is closed, pet cock *B* opened. The air reservoir is filled by operating the compression pump *L;* pet cock *B* is then closed. After preparing the site of operation in the usual manner pet cock *C* is opened, ink applied to the tracer *E* and the kymograph started by elevating the release lever *K*. The pneumothorax needle *F* is forced carefully into the pleural cavity and as entrance occurs the tracer *E* will swing to the left (to the right if the

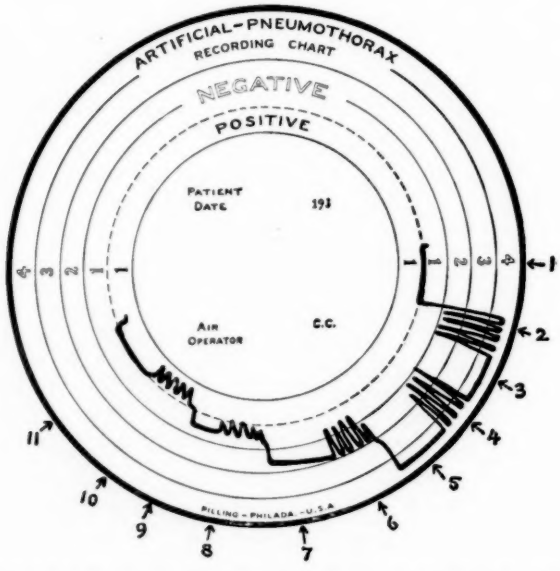


Fig. 2.—The irregular line represents different stages in treatment. During periods 1, 3, 5, 7, 9, pet cock *C* was closed. Periods 2, 4, 6, 8 show "negative" respiratory movements recorded while pet cock *A* was closed and pet cock *C* open. Period 6 indicates an approach to positive pressure and period 9 shows that positive pressure has occurred. The operation was terminated during period 11.

pressure is positive) and the degree of pressure will be recorded on the chart *G*. Pet cock *C* is closed and the lever on pet cock *A* is opened to the first numeral on the dial. As this occurs air will pass into the pleural cavity, the rate and amount being shown on the gauge *H*. The rate is increased by moving the lever on pet cock *A* to numerals 2, 3 or 4. At intervals pet cock *A* is closed and *C* is opened in order to determine the approach to positive thoracic pressure. When the tracer *E* passes to the right of the dotted line on the chart, the indication is that positive pressure has occurred. At this point treatment should be discontinued by closing the release lever *K*, pet cocks *A* and *C* and withdrawing the pneumothorax needle *F*. The total amount of air that has entered the chest is determined by subtracting the number indicated by the hand on gauge *H* from the figure noted at the beginning of the operation. This is

written on the chart, with the date, patient's name and that of the operator. About once every three weeks for antiseptic purposes, 2 c.c. of alcohol should be dropped into the air filter *M*.

The chief features of the instrument are the exclusion of manometers, glass jars, and stoppers and the use of a device for recording variations in the degree of thoracic pressure. These simplify operation and reduce the source of error.

The instrument is manufactured by the George P. Pilling and Son Company, Philadelphia, Pa.

A SIMPLE CLINICAL PROCEDURE FOR THE DETERMINATION OF UREA IN URINE BY MEANS OF HYDROLYSIS*

By S. L. Leiboff, A.B., M.A., and Bernard S. Kahn, A.B., M.D.,
New York City, N. Y.

IN 1929 we[1] described a method for the determination of urea in blood by digesting the protein-free blood filtrate under pressure in a closed tube.† We have now extended the use of this method to the quantitative determination of urea in urine. The details of the procedure are here described:

Principle.—The ammonia is removed from the urine with permutit according to the procedure of Folin and Bell.[2] The ammonia-free urine is digested under pressure in an acid medium for ten minutes. The resulting ammonium salt is nesslerized directly in the digestion tube and compared in the colorimeter to a known standard.

Procedure.—Three c.c. of urine are placed in a 200 c.c. Erlenmyer flask to which are added 3 to 4 grams of permutit and 27 c.c. of water. The mixture is shaken for five minutes and filtered through filter paper. Five c.c. of the filtrate are diluted with water to 100 c.c. in a volumetric flask. Five c.c. of this diluted filtrate (1:200 dilution) are placed in a urea pressure tube to which is added 1 c.c. of approximately normal H_2SO_4. The stopper is rinsed with 1 c.c. of water and tightened in the neck of the tube. The tube is then placed in an oil-bath which is heated at 145°-150° C. for ten minutes. The tube is removed from the oil-bath and cooled. It may be cooled immediately under running tap water without any danger of breaking the tube. About 15 c.c. of water are then added, followed by 3 c.c. of modified Nessler solution.[3] Water is added to the 25 c.c. mark and the contents well mixed. It is then compared in the colorimeter against a standard containing a known amount of ammonia.

The standard is the same as used for the determination of urea in blood. It contains 0.3 mm. of nitrogen in 5 c.c. of solution. It is prepared by placing

*From the Biochemical Laboratory of Lebanon Hospital.
Received for publication, May 11, 1931.

†The pressure tubes and a special oil-bath may be obtained from Eimer and Amend, New York.

5 c.c. of the standard ammonium sulphate in a 100 c.c. volumetric flask containing about two-thirds its volume of water. To this are added 12 c.c. of Nessler solution and water to the 100 c.c. mark.

The standard is placed in the colorimeter at 20 mm. If the color of the unknown is much deeper than that of the standard due to a greater concentration of ammonia, a portion of the unknown solution is transferred to a narrow tall graduated cylinder and diluted with dilute (1:10) Nessler solution until the color of the unknown approximately matches that of the standard.

Calculation of Results:

$$\frac{6000}{R} \times \text{Dilution} = \text{Mg. nitrogen in 100 c.c. of urine.}$$

R = Reading of unknown.
Dilution = Dilution of Nesslerized solution.

This method of hydrolysis of urea also breaks down other substances than urea and therefore gives slightly high results. We therefore do not advocate the use of this method for determination of urea in urine when absolute values are desired, such as, for example, when studying the nitrogen partition. However, when a nitrogen partition is to be made, in spite of the somewhat higher values, we prefer this method to the methods using urease hydrolysis and we find it satisfactory for clinical purposes.

The pressure tubes, after use, are washed with dilute nitric acid and thoroughly rinsed with water. Omission to do this, will leave some Nessler reagent in the tube which will produce cloudy solutions. The tubes after being thoroughly washed are placed in a rack with their mouths down in such a manner that the stoppers are held loosely halfway in the aubes, thus allowing the water to drain.

Nessler solution should never be added to a warm solution and preferably is to be added rapidly. When these precautions are observed a clear solution will always be obtained.

REFERENCES

1. Leiboff, S. L., and Kahn, Bernard S.: A Rapid and Accurate Method for the Determination of Urea in Blood, J. Biol. Chem. **83**: 347, 1929.
2. Folin, O., and Bell, R. D.: Application of a New Reagent for the Separation of Ammonia. I. The Colorimetric Determination of Ammonia in Urine, J. Biol. Chem. **29**: 329, 1917.
3. Koch, F. C., and McMeekin, T. L.: New Direct Nesslerization Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. **46**: 2066, 1924.

Dr. A. J. Rongy, Fellow, Lebanon Hospital.

AN IMPROVED MICRO KJELDAHL METHOD*

By J. W. Cavett, Ph.D., Minneapolis, Minn.

OWING to the necessity for a Kjeldahl method that could be used to analyze small quantities of nitrogen which were present in relatively large volumes of liquid and still maintain an accuracy similar to that of the macro Kjeldahl, the following method was devised. This method is capable of

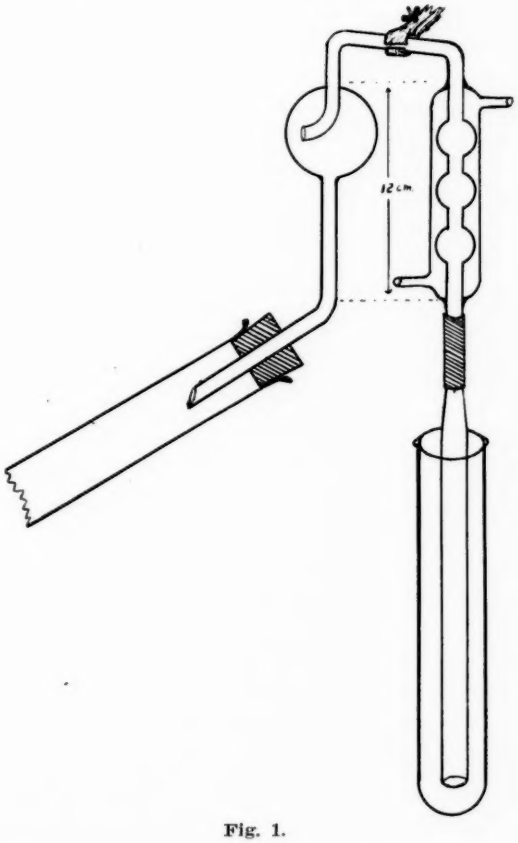


Fig. 1.

determining 0.5 mg. to 14 mg. of nitrogen with an accuracy of 0.006 mg. or less. Bumping and sucking back have been eliminated due to modifications of the apparatus. Also the chance for a leak during distillation has been greatly reduced and the whole apparatus may be easily cleaned as it is all glass. The distillation period has been shortened to ten minutes and the titration is carried out in a manner which increases its accuracy.

*From the Laboratory of Physiological Chemistry, University of Minnesota.
Received for publication, April 13, 1931.

METHOD

The sample to be analyzed is placed in a 300 c.c. Kjeldahl flask and 4 c.c. of concentrated sulphuric acid and a few drops of 5 per cent copper sulphate solution added. (A half gram of sodium sulphate or a few drops of H_2O_2 increases the speed of digestion.) The digestion is carried out in a hood. After digestion is complete and the flask has cooled, 40 c.c. of distilled water is added. Four glass beads and about 10 particles of 20 mesh zinc are added to prevent bumping. The mixture is then underlaid with 12 c.c. of 1 to 1 carbonate-free sodium hydroxide. The flask is connected by a new *soft* rubber stopper to the special Kjeldahl bulb which is suspended from a ring-stand by a burette clamp. The special Kjeldahl bulb including a small condenser is shown in Fig. 1. A 1½ cm. glass tube constricted at the upper end and 25 cm. long is attached to the lower part of the condenser by a piece of rubber tubing. This glass tube extends into a 22 × 3 cm. test tube which contains 10 c.c. of standard acid and a drop of methyl red solution. The test tube is held by a clamp so that the delivery tube extends to the bottom at first but after the distillation is in progress it is kept about 1-2 cm. below the surface. The standard acid used is N/10 or N/50 depending on the amount of nitrogen in the sample being analyzed. After all connections are complete, the flask is rotated thus, mixing the solution with the NaOH. Twenty-five c.c. of water is then distilled over.

The titration is always carried out with N/50 carbonate free sodium hydroxide. With the smaller quantities of nitrogen, 0.5 to 2.8 mg., when N/50 standard acid is used the titration is made with a 10 c.c. burette calibrated to 0.05 c.c., otherwise an ordinary accurately calibrated 50 c.c. burette is used. The acid in the test tube is stirred during the titration by bubbling air which has passed through weak acid and alkali through it. By this method of stirring the solution during the titration, one can easily determine an end-point to within 0.02 c.c. of N/50 NaOH when using the 10 c.c. burette. Less time is required than if the solution is stirred with a stirring rod or shaking and the end-point is easier to read.

SUMMARY

The Kjeldahl bulb is fused on to the condenser giving an apparatus which is compact, easy to clean and eliminating possibility of leaks in connections. The use of smaller quantities of reagents and the presence of glass beads and 20 mesh zinc eliminates bumping. The sucking back is avoided by use of a large delivery tube and with moderate care a determination is never lost. The method has the advantage that a number of determinations can be run at once having an accuracy similar to the macro Kjeldahl on a sample almost as small as is ordinarily used for the micro Kjeldahl.

NOTE: The special Kjeldahl apparatus may be obtained from Arthur H. Thomas Co., Philadelphia.

A NEW AND SIMPLIFIED TECHNIC OF SURGICAL PHOTOGRAPHY*

By L. F. Pierce, Ph.D., Carmel, Calif.

FOR a considerable period, photography of operative surgery has been a topic of interest. Frequent articles appear setting forth various items of technic and equipment. The writer pleads no interest beyond that of the photographic amateur who is interested in any discussion tending to raise the level of teaching scientific subjects.

Observation of two reels of surgical movies brought a curious thought to mind with the result that the reels were run again with a stop watch. The two reels of fifteen minute length checked within forty seconds of each other in that the field of operations was masked for a total of six minutes by the hands of either the surgeon or his assistant, a total loss of 40 per cent in the picture. When we recall that good moving picture equipment complete for such work cannot be had for less than approximately three to six hundred dollars with an actual outlay of about eight to twelve dollars for film used to make a picture of six to ten minutes' duration, the cost item mounts rapidly enough that a 40 per cent loss is appreciable.

It is the understanding of the writer that actual surgical technic is very definitely taught to the medical student and later to the graduate. There is of course justification for moving picture records of a great master doing each classical operation. From a teaching standpoint, there is even justification for multiplication of these pictures to show the various modifications of these technical items. But for the daily photographic need of the surgeon, it would appear that his real need is to have a permanent photographic record of the unusual or anomalous details which come to light at the operating table. If these details can be readily recorded without the aid of a professional photographer and preferably by a nurse in a simple manner with a minimum of special equipment and at a nominal cost, it would seem that a desirable end had been reached.

For this work, film packs or plates must generally be avoided in favor of roll film. The lens must be capable of working at large aperture to shorten exposure. The focal length must be short to obtain maximum focal depth and avoid extremely critical focusing. The shutter should be of the iris type rather than focal plane in order to use the camera for making records of gross specimens with extremely small aperture and long automatic exposure and thus obtain fine detail at different levels. Film stock should be obtainable at low cost from any good camera supply store and the camera should be simply and reliably made and capable of being loaded as often as need be by a person having but a slight knowledge of a camera. Finally, the finished negative should be capable of enlargement without loss of detail to five by

*From the Grace Deere Velie Metabolic Clinic.
Received for publication, May 18, 1931.

seven inches for record and to lantern slide size for projection. If in addition to this, the camera can be used for amusement, all the better for the individual who gets pleasure from the pictorial hobby.

Until a few months ago, there has been but one camera in the market which even began to meet these requirements. Because, however, of its usual focal plane shutter equipment, it is not desirable for slow exposures at small aperture of gross specimens. Further, the cost has been considerable, the film is not readily obtainable all over the country and loading the camera is, to put it mildly, formidable.

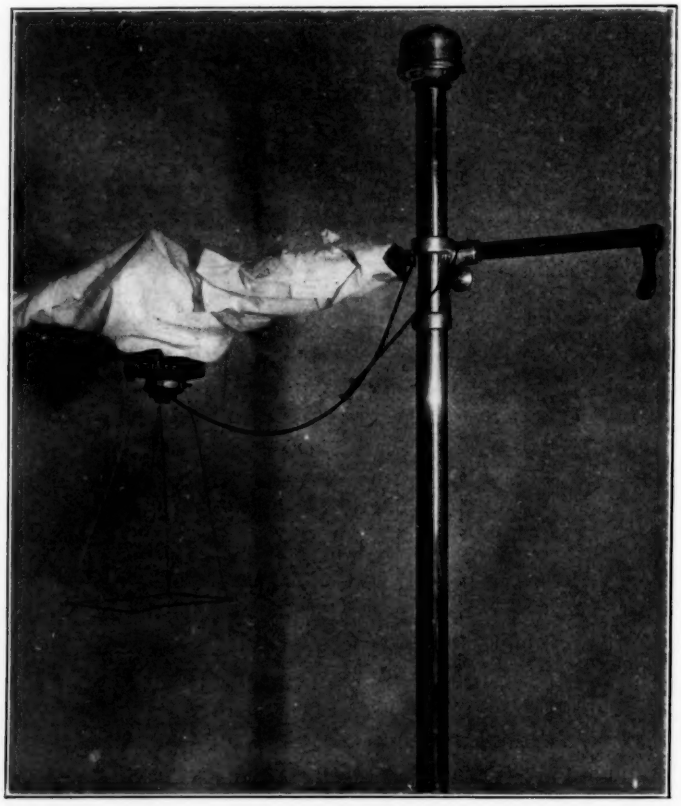


Fig. 1.—Camera and pyramid iconometer as supported on lamp standard.

The optical firm of Carl Zeiss, Inc., has but recently produced a camera which really meets these needs in every way and at such a cost that total equipment for taking a rapid succession of stills of this kind may be assembled for well under a hundred dollars which will "cover" an average operation with a film cost of fifty-five cents and a slide cost of around four dollars if wanted.

Accurate location of the field of view is a problem, in that it is undesirable to have the camera operator leaning over the patient to look squarely down. Mirrors would add to complexity and confusion. The problem is met satisfactorily by a readaptation of the iconometer commonly used photo-

graphically on the continent. A felt padded metal collar with a constriction fitting is clamped on the shutter barrel of the "Kolibri" camera. From this four wires extend downward at angles to join a rectangular wire frame seven and one-half by ten inches which is conveniently located at a distance of thirteen inches from the lens. The lens being the tessar f. 3.5 and fitted with the 1 × 24 proxar auxiliary and the shutter at maximum extension the plane of focus is at twenty inches. The frame must therefore approach to a distance of seven inches from the desired object in the wound. An ordinary six inch hemostat held vertically by the operator makes estimation of the last inch a simple matter and the frame is at no time in danger of touching anything. The frame is preferably wiped with alcohol before use. The entire outfit is suspended by means of a rubber covered four finger condenser clamp pointing down from the horizontal arm of almost any convenient therapeutic lamp standard readily found in the hospital. The actual lamp is, of course, dis-

Fig. 2.—Supravaginal amputation of fibroid uterus. Tumor delivered and clamps applied to broad ligaments.

mounted. The entire set-up is shown in Fig. 1. Thus at the call of the surgeon who steps to one side by six to twelve inches, the stand is wheeled into place, the camera lowered to the proper distance, maneuvered to center, the shutter tripped by means of a long cable release and the entire outfit pulled clear. The time for all this is not in excess of a second and a half.

If the surgeon uses a bit of sterile vaseline and free powdering of the hands with sterile zinc stearate, visibility of the hands is much enhanced.

In view of the many colors encountered which are not registered on ordinary orthochromatic film, panchromatic emulsion is called for and may be readily obtained at any photo supply counter through G. Gennert, Inc., of New York, the agents of the Imperial Dry Plate Works of England. At present no firm in this country is making film of this kind in the ordinary roll stock. Use of this emulsion gives excellent color separation without the use of filters. The speed of the emulsion is "par." Processing is simple if we emphasize to our finisher that the film must be handled either in entire dark-

ness or under the Wratten safelight. In our work we found that the fine grain borax developer formula of Eastman gave the best results by far and we used it in tray strength for ten minutes at 65° F. A retail cost of fifty-five cents per roll of sixteen exposures is not excessive.

Exposures for this work must be measured with great care. The exposure meter of Dr. E. Meyer is very good and gives satisfactory results. We found however that we were able to expose preferentially to emphasize a given part of the wound by the use of the electric photometer of Bell and Howell. The instrument is simple and most exact. Use of such a device makes the difference between exact measurement of actinic light and pure guesswork.

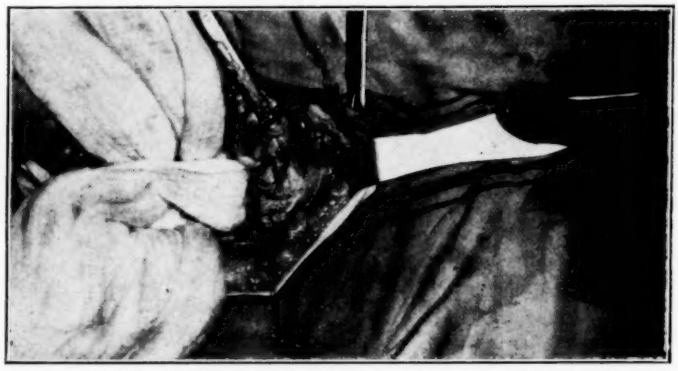


Fig. 3.—Stump in abdominal wound after removal of uterus containing fibroid.

In our work given herewith in plates 2 and 3 exposures of $\frac{1}{50}$ second at f. 3.5 were used with the regular nonshadow lamp found above the table. Slight variations were made for preferential emphasis. Repeated trials were made with different operators and in every case the concern of the surgeon gave way to pleased amazement and perfect cooperation at the end of the third picture. The entire time required for fourteen pictures during an hysterectomy was seventeen seconds which in no way can be said to interfere with the course of the operation.

In conclusion, we wish to repeat that our entire object has been to present a photographic technic for surgery which is simple, exact, comprehensive and finally, most economical.

Drs. H. C. Oatman, M. M. Doria, and R. J. Pickard are thanked for the kindly aid and cooperation which made this work possible.

A NEW PETRI DISH HOLDER FOR COUNTING AND FISHING COLONIES*

By Nicholas Kopeloff, Ph.D., and Nathan Blackman, B.S.,
New York, N. Y.

TRADITION dictates the counting and fishing of bacterial colonies as carried out in various research, diagnostic, dairy, and public health laboratories. Little progress has been made in counting bacteria beyond the introduction of the glass counting plates devised by Jeffer, Wolfhuegel and others. The accepted procedure is to superimpose the etched glass counting plate on the bottom half of the Petri dish. Then, with the aid of a hand magnifier, the colonies are counted against a dark background such as a black table top for example, or the Petri dish is held at eye level against a source of light, daylight or artificial. When the Petri dish is held at eye level the colonies in the depths of the medium are more visible and the counting process becomes more accurate. However, the hands tire rather easily in this awkward position and one cannot employ a mechanical counter because both hands are already occupied. While there is an expensive apparatus on the market that permits free use of the hands its magnifying lens fails to bring out small colonies with sufficient clarity.

Similarly, in fishing colonies the attempt is usually made to work under aseptic conditions which frequently necessitates artificial illumination from above. In any event it is difficult to see clearly into the depths of the medium and often there is great hardship in fishing from the edges of given colonies as is necessary in microbial dissociation studies.

A simple Petri dish holder devised by one of us (Blackman) is herewith described. It may be attached to the upright stem of an ordinary iron support. The holder is a metal frame which holds the Petri dish in place at any height and at any angle, thus permitting free use of the hands. Plate 1 is a photograph of the front view and Plate 2 is a rear view.

The specifications of the Petri dish holder are as follows: A square piece of galvanized iron sheeting 5 inches wide and $\frac{1}{32}$ of an inch thick has an edge folded at right angles to the depth of $\frac{3}{4}$ of an inch. It is painted black. A round hole exactly the size of a Petri dish is cut in the metal sheet. A Jeffer's glass counting plate is then fastened on the inside of the frame. Four $\frac{5}{8}$ inch stay springs (*A*) at the rim of the hole hold the Petri dish in place. The edges of the stay springs are bent up to hold the bottom half of the Petri dish in place. Two tension springs (*B*) $1\frac{3}{4}$ of an inch on a $\frac{3}{4}$ inch post clamp the Petri dish in place. The metal frame is moved up and down on the upright

*From the Department of Bacteriology, Psychiatric Institute and Hospital, New York City.

Received for publication, May 6, 1931.

(Copyright, 1931, By Nicholas Kopeloff, New York City.)

stem of an iron support by means of a slide joint (*C*). A tight fitting swivel joint (*D*) allows the frame to be tipped to any desired angle.

The construction of this Petri dish holder is simple and the cost of materials almost negligible.* The Petri dishes are firmly fixed.

The advantages of this Petri dish holder may be thus briefly summarized:

1. The hands are left free for use in handling a mechanical counter, fishing, etc.

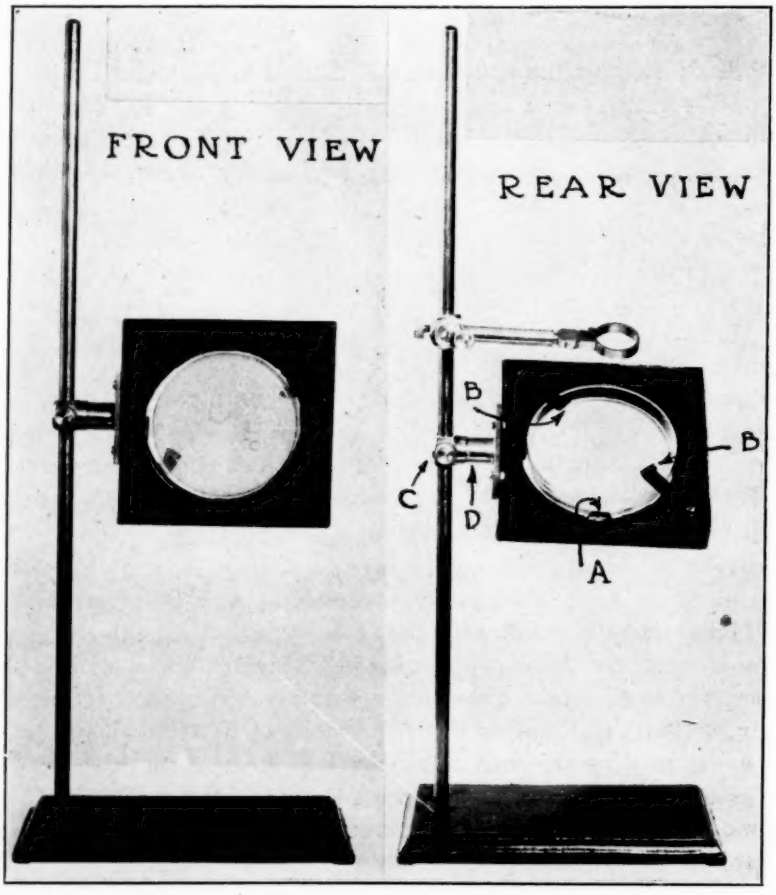


Fig. 1. Fig. 2.

2. Petri dishes are clamped in such a way that either daylight or artificial illumination is allowed to penetrate the depths of the medium.

3. Counting or fishing can be done at any desired angle or eye level. When fishing the frame is tilted at an angle of 45° and a microscope lamp with blue glass filter is used.

4. There is no movement of the Petri dish to make for inaccuracy.

In order to facilitate direct examination of the colonies with a magnifier one of us (Blackman) has devised an adjustable holder for the magnifying

*Thanks are due Mr. E. L. Phelan for his skillful execution of the design.

lens. This also is attached to the upright of an iron support. Plate 3 is a front view photograph.

The magnifying lens is held in a brass holder consisting of the following parts. The binding screw (*A*) permits the holder to be fixed at any level. A stud (*B*) has a tension screw which holds another stud (*C*) in place at right angles to it. Stud *C* is moved back and forth on thread of screw (*D*) which

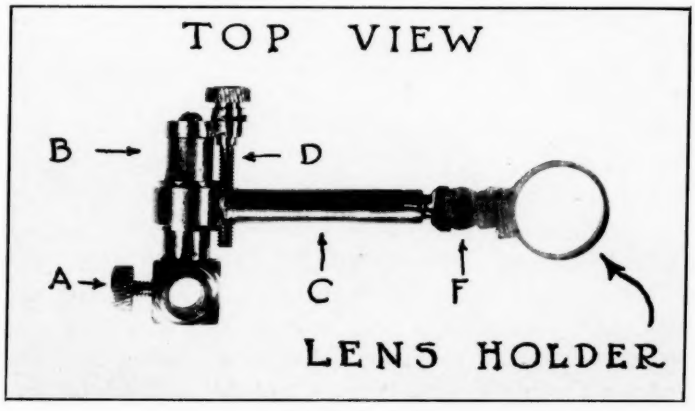


Fig. 3.

facilitates fine adjustment. A friction joint within stud *C* supports the lens holder (*E*) which has a knuckle joint (*F*) permitting it to move in a semicircle.

SUMMARY

An adjustable Petri dish holder and magnifying lens holder are described which are of economical construction. They leave the hands free for the use of a mechanical counter or for fishing colonies. Daylight or artificial light may be employed.

A SIMPLE PRESUMPTIVE TEST FOR AGGLUTINATION WITH ORGANISMS OF THE ABORTUS-MELITENSIS GROUP*†

By Ruth Gilbert, M.D., and Marion B. Coleman, B.S., Albany, N. Y.

THE constantly increasing recognition and, possibly, incidence of undulant fever necessitate a careful evaluation of the agglutination reaction upon which the diagnosis is at present so dependent. The specificity of this test with organisms of the abortus-melitensis group is obviously determined by results obtained in the examination of sera from (1) cases of undulant fever, (2) cases of other disease, particularly those accompanied by a febrile reaction, and (3) normal individuals. The incidence of such reactions has already been reported by many observers,[1-17] but only a few have had the opportunity to examine large numbers of specimens. The potential value of testing with B. abortus all sera submitted for the complement-fixation test for syphilis prompted the development of a practical and economical technic for this purpose. The macroscopic-slide method of Huddleson[18] was first considered, but while this gives results very quickly, the testing of five dilutions of each serum requires the same number of pipettes and almost as much time as the routine macroscopic-tube method. The following procedure has, in our experience, proved most economical of both time and glassware.

Technic of the Presumptive Test.—At the time sera for the complement-fixation or other tests are measured, 0.01 c.c. is pipetted into a properly labeled 11 by 75 mm. tube. These tubes are arranged in serial order in racks and empty tubes placed behind them. With a 10 c.c. pipette graduated in tenths, 0.6 c.c. of a suspension of B. abortus (killed by heating at from 60 to 65° C. and preserved by addition of 0.1 per cent formalin) of one-half the density of barium sulphate standard[19] No. 3 are measured into both the empty tubes and those containing serum, thus providing a serum dilution of 1:60 in the latter. The racks are shaken vigorously to mix the serum and suspension of organisms. It was found by calculation that a 19 gauge needle, from which the tip has been removed by filing, when attached to the barrel of a 1 c.c. syringe fitted with a rubber bulb, delivers approximately 0.019 c.c. per drop. Therefore, by the additon in this way of two drops of the 1:60 serum dilution from the tube in the front row to 0.6 c.c. of suspension in the tube behind it, a dilution of approximately 1:900 is obtained. Thus, a low dilution is provided for weakly reacting sera, as well as a dilution sufficiently high to be above the range of prezone reactions. After the two drops are delivered, the excess of the 1:60 dilution is replaced in the tube from which it was taken.‡ Before the next specimen is diluted, the syringe and needle are rinsed first with tap water and then with 0.85 per cent salt solution, both of which are contained in large jars so that only a negligible amount of serum is carried from one series of dilutions to the next. After being shaken, the tests are placed in a 55° C. in-

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

Received for publication, May 1, 1931.

†Preliminary results presented at the meeting of the American Society of Clinical Pathologists, Detroit, Michigan, June 21, 1930.

‡When the presumptive procedure was first adopted, dilutions of 1:20 and 1:320 were tested. The change was made because reactions in dilutions lower than 1:80 are of questionable significance and the prezone has been found, in at least one instance, to extend through the 1:320 dilution.

cubator for four hours, and left in the refrigerator overnight before being read. Whenever a reaction is observed in either the 1:60 or 1:900 dilution or both, the serum is retested in dilutions ranging from 1:10 through 1:5000.

This simple procedure made it possible to examine 81,848 sera received for the complement-fixation test for syphilis. Three hundred and sixty-nine, or 0.4 per cent of these, when retested with the usual amounts of serum, were found to react in a 1:80 or higher dilution. While most of the histories refer to the symptomatology of syphilis, presumably only a small proportion of such specimens are from cases of febrile disease and many of the reactions are no doubt due to previous infections with organisms of the abortus-melitensis group. In 44 instances or 0.05 per cent of those in which specimens were tested, a diagnosis of undulant fever was indicated. These findings are in sharp contrast to the results of the examination of 1,186 sera submitted for the agglutination test with B. typhosus. Of this number, 70 or 5.9 per cent gave reactions in a 1:80 or higher dilution and 57 or 4.8 per cent were from patients found to have symptoms of undulant fever. Furthermore, in the examination of 1,255 specimens accompanied by a request for the test with B. abortus, 145 or 11.5 per cent gave reactions and 102 or 8.1 per cent were from probable cases of undulant fever. A summary of the reactions obtained with B. abortus in a 1:80 or higher dilution, correlating the degree of agglutination and the clinical manifestations, is given in Tables I, II, and III.

TABLE I

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 369 SERA WHICH AGGLUTINATED B. ABORTUS IN A 1:80 OR HIGHER DILUTION, AMONG 81,848 SPECIMENS SUBMITTED FOR THE COMPLEMENT-FIXATION TEST FOR SYPHILIS

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1:10,000	1:5000	1:2500	1:1200	1:640	1:320	1:160	1:80
Sera giving reactions	369	1	3	5	11	32	49	76	192
Undulant fever definitely diagnosed	14	1	1	1	2	2	4	2	1
Symptoms of undulant fever; definite diagnosis not made	30			1	3	6	4	6	10
No history of undulant fever reported by physician	69			1	1	5	12	15	35
No information or insufficient for drawing conclusions	256		2	2	5	19	29	53	146

The infrequency of agglutination reactions with B. abortus in 1:80 or higher dilutions of sera from individuals with no history of undulant fever confirms previous reports on the diagnostic significance of this test. The relatively large number of instances in which a diagnosis of undulant fever has been made as a result of testing for agglutination with B. abortus sera submitted to be examined for evidence of typhoid fever or syphilis, i.e., 101 instances in which the possibility of undulant fever had not been considered by the attending physician before the test was made, not only justifies the procedure, but indicates that the syndrome of infection with organisms of the abortus-melitensis group is still frequently unrecognized.

TABLE II

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 70 SERA WHICH AGGLUTINATED B. ABORTUS IN A 1:80 OR HIGHER DILUTION, AMONG 1,186 SPECIMENS SUBMITTED FOR THE AGGLUTINATION TEST WITH B. TYPHOSUS

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1:10,000	1:5000	1:2500	1:1200	1:640	1:320	1:160	1:80
Sera giving reactions	70	2	5	7	15	18	12	8	3
Undulant fever definitely diagnosed	44	2	5	4	11	10	7	4	1
Symptoms of undulant fever; definite diagnosis not made	13			1	3	3	4	1	1
No history of undulant fever reported by physician	1					1			
No information or insufficient for drawing conclusions	12			2	1	4	1	3	1

TABLE III

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 145 SERA WHICH AGGLUTINATED B. ABORTUS IN A 1:80 OR HIGHER DILUTION, AMONG 1,255 SPECIMENS SUBMITTED FOR THIS TEST

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1:10,000	1:5000	1:2500	1:100	1:640	1:320	1:160	1:80
Sera giving reactions	145	4	5	12	21	46	22	19	16
Undulant fever definitely diagnosed	81	4	3	5	17	27	16	7	2
Symptoms of undulant fever; definite diagnosis not made	21			5	2	8	2	1	3
No history of undulant fever reported by physician	3				1			1	1
No information or insufficient for drawing conclusions	40		2	2	2	10	4	10	10

REFERENCES

1. Bellinger, G. C., and Levin, W.: Undulant or Malta Fever in Oregon, Northwest Med. **28:** 9, 1929. (Cited in J. A. M. A. **92:** 844, 1929.)
2. Carpenter, C. M., Boak, Ruth, and Chapman, O. D.: The Significance of Brucella Abortus Agglutinins in Human Sera, J. Immunol. **17:** 65, 1929.
3. Evans, A. C.: Malta Fever. Cattle Suggested as a Possible Source of Infection, Following a Serological Study of Human Serums, U. S. Pub. Health Rep. **39:** 501, 1924. Some Unsolved Undulant Fever Problems. In: American Public Health Association, Undulant Fever Symposium, 1929, p. 29, New York City.
4. Gilbert, Ruth, and Coleman, M. B.: Recent Cases of Undulant Fever in New York State, J. Infect. Dis. **43:** 273, 1928.
5. Giordano, A. S., and Ableson, Marjorie: Brucella Abortus Infection in Man. A Serologic Survey, J. A. M. A. **92:** 198, 1929.
6. Harrison, H., and Wilson, G. S.: The Possible Existence in This Country of Disease Due to Infection With Brucella Abortus, Lancet **215:** 1338, 1928.
7. King, M. J., and Caldwell, D. W.: Brucella Abortus in Milk Supply as a Source of Agglutinins in Human Sera, Am. J. Med. Sc. **178:** 115, 1929.
8. Kristensen, Martin: Untersuchungen über die Rolle des Bangschen Abortbazillus als menschenpathogenen Mikroben, Centralbl. f. Bakteriol. Orig. **108:** 89, 1928.
9. Kristensen, Martin, and Holm, Per: Bakteriologische und statistische Untersuchungen über Febris undulans in Dänemark, Zentralbl. f. Bakteriol. Orig. **112:** 281, 1929.
10. McKay, A. L., and McNabb, A. L.: Undulant Fever in Ontario, Canadian Pub. Health J. **20:** 85, 1929. (Cited in Bull. Hyg. **4:** 514, 1929.)
11. McAlpine, J. G., and Mickle, F. L.: Bacterium Abortus Infection in Man. The Results of the Agglutination Test Applied to More Than 10,000 Human Sera, Am. J. Pub. Health **18:** 609, 1928.

Further Observations on Human Infection With Brucella Abortus. In: American Public Health Association, Undulant Fever Symposium, 1929, p. 12, New York City.
12. Madsen, T.: La fievre ondulante à B. abortus du Danemark, Bull. Office Internat. d'Hyg. Pub. **20:** 1395, 1928. (Cited in Bull. Hyg. **4:** 512, 1929.)
13. Orr, P. F., and Huddleson, I. F.: A Further Epidemiological Study of Undulant Fever in Michigan. In: American Public Health Association, Undulant Fever Symposium, 1929, p. 34, New York City.
14. Poppe, K.: Die Bang-Infektion des Menschen, Deutsch. tierärztl. Wchnschr. **36:** 781. 1928. (Cited in Centralbl. f. Bakteriol. Ref. **93:** 493, 1929.)
15. van der Hoeden, J.: Over febris undulans by den mensch, veroorzaakt door bacterium abortus infectiosi bovum Bang, Ned. T. voor Geneeskunde **2:** 490, 1928. (Cited in Zentralbl. f. Bakteriol. Ref. **95:** 162, 1929.)
16. Weigmann, F.: Über menschliche Infektionen mit Bact. abortus Bang und ihre Verbreitung in Schleswig-Holstein, Klin. Wchnschr. **8:** 351, 1929.
17. Welsh, M. F.: The Examination of 2,433 Human Sera for Agglutinins of Brucella Abortus, J. Immunol. **17:** 285, 1929.
18. Huddleson, I. F., and Abell, Elizabeth: Rapid Macroscopic Agglutination for the Serum Diagnosis of Bang's Abortion Disease, J. Infect. Dis. **42:** 242, 1928.
19. McFarland, Joseph: The Nephelometer: an Instrument for Estimating the Number of Bacteria in Suspensions Used for Calculating the Opsonic Index and for Vaccines, J. A. M. A. **49:** 1176, 1907.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PNEUMOCOCCI, Rapid Method of Typing, Brown, M. H. Am. J. Pub. Health 21: 669, 1931.

1. A representative sample of sputum should be obtained, if necessary, waiting until the patient expectorates material directly from the bronchial tract instead of saliva or pharyngeal secretions. A surprisingly small amount of actual sputum is necessary. The sample should be obtained in a clean sterile container, free from any preservative.

2. Wash the sputum at least three times by swirling the mass of sputum around in sterile saline contained in three separate Petri dishes. This tends to remove extraneous microorganisms. Following this, the sputum is thoroughly emulsified in saline, and in order to facilitate this, a 5 c.c. syringe and a needle of large caliber are very useful. At least 1 c.c. of emulsified sputum is then injected intraperitoneally into a mouse, using a needle of small caliber. As a matter of routine, two mice are injected.

3. In three hours a peritoneal puncture is performed, using a capillary pipette made by drawing out a piece of small glass tubing in a flame. The finer the pipette, the more satisfactory it is for this purpose. Grasp the mouse by the loose skin of the neck between the thumb and index finger, with the back of the mouse across the palmar surface of the hand, and made secure by looping the tail between the ring and little fingers. The abdomen of the mouse being thus well exposed, the capillary pipette is passed into the peritoneal cavity and by capillary traction fluid will be seen extending into the pipette. The pipette is then removed and the mouse is kept for further observation.

4. The surface of a well-cleaned glass slide is divided into four sections, using a glass pencil. On each of these four sections is placed a small drop of the peritoneal exudate from the pipette. With the first of the four drops of peritoneal exudate is mixed a drop of saline which is then spread thinly and allowed to dry. To the second drop of the exudate, a drop of Type I agglutinating serum diluted 1 in 10 is added and the mixture is spread thinly and allowed to dry. Similarly, the third drop of exudate is mixed with a drop of Type II agglutinating serum, diluted 1 in 10, and is spread thinly and allowed to dry, and to the last drop of exudate a drop of Type III agglutinating serum diluted 1 in 5, is added and the mixture is spread thinly and allowed to dry. The films are then fixed by passing the slide two or three times over a moderate flame. (For convenience one usually labels each of the above four sections as Sal., I, II, III with red pencil.)

5. The slide is then stained for one minute with basic fuchsin stain. The stain, which should be fresh, is made by adding 10 c.c. of a saturated alcoholic solution of basic fuchsin to 90 c.c. of distilled water, and filtered through paper. (The saturated alcoholic solution is made by adding 10 gm. of basic fuchsin to 100 c.c. of absolute alcohol.) Other stains such as methyl violet may be used, but basic fuchsin has been found to be the most satisfactory. Wash off the stain gently with water, blot dry and examine under an oil immersion lens. The saline suspension is examined first and serves as a control. The character of the microorganisms is then determined. Frequently a pure culture of a lance-shaped diplococcus is found. The other sections are examined in sequence, and definite clumping of lance-shaped diplococci in one of the sections serves to denote the type. There may be many clumps consisting of only a few organisms or a few large clumps covering several microscopic fields. In examining for clumps one should not be misled by microorganisms, such as gram-negative cocci, which tend to occur in clumps, or the grape-like clumps of staphylococci. These may be distinguished by being round or coccoid in shape in contrast to the lance-shaped diplococcus of pneumonia. The so-called capsule may be quite marked, especially in animal exudates such as obtained from the peritoneum of the mouse. Type III pneumococci usually show very

marked capsules, often so much so that the organisms themselves are seen with difficulty and the clumps are not usually packed so tightly together as are Type I or Type II organisms. Further, in a clump of Type III pneumococci one may see a very fine thread-like matrix extending from organisms to organism.

Usually the sputum is well digested in the peritoneal cavity of the mouse within three hours, especially in Type III cases, but occasionally one may be required to repeat the peritoneal puncture after a lapse of four or even six hours in order to obtain a satisfactory result. In our experience a sputum in which a satisfactory typing was not obtained after six hours was either free of a fixed type of pneumococcus or contained some other organisms such as a streptococcus.

6. The mouse almost invariably survives the peritoneal puncture, but, if the organism is virulent, the mouse dies in from eighteen to twenty-four hours. The peritoneum is then opened under aseptic precautions and the contents are washed out with 3 to 4 c.c. of saline. The washings are centrifuged, first at very low speed in order to throw down any blood cells. The supernatant fluid is drawn off and centrifuged at high speed to throw down the microorganisms.

A "ring test" or precipitin test is performed by floating about 0.1 c.c. of the supernatant over an equal quantity of agglutinating serum, diluted 1 in 10. At the point of contact of serum and fluid a fluffy white ring appears almost immediately in the tube containing the serum of the corresponding type. The standard macroscopic agglutination test may be made with the centrifuged sediment. The latter two tests serve as a check on the rapid method, but in our experience the "ring test" is more readily and accurately interpreted. A broth culture may be made from the heart's blood of the mouse for purposes of further examination, and usually a pure culture of the pneumococcus is obtained from this source.

In a very early case of pneumonia, the first strain isolated may not be one of the fixed types so that it is often advisable to obtain another sample of sputum and repeat the search. In this way a Type I, II, or III, which otherwise would have been missed, may be obtained.

TISSUE STAIN: Modification of Mallory's Triple Stain, Krichesky, B. Stain Tech. 6: 97, 1931.

Tissues fixed in Bouin's or Zenker's fixing fluids have given good results when used with this modification which is as follows:

I. *Staining solutions:*

Solution 1.		
Acid fuchsin (dye content 62 per cent)	0.25	gm.
Water	100	c.c.
Solution 2.		
A. Anilin blue	2.0	gm.
Water	100	c.c.
B. Orange G (dye content 83 per cent)	1.0	gm.
Water	100	c.c.
C. Phosphomolybdic acid 1 per cent	100	c.c.

Solutions A, B, and C should be kept in separate bottles because the mixture deteriorates on standing. When ready for use, solution 2 is made up of equal parts A, B, and C.

II. *Procedure:*

If sections are 10 microns or less in thickness, hydrate by passing through the descending series of alcohols to 70 per cent alcohol and then directly into water for five minutes. Proceed with the steps as follows:

1. Transfer sections to solution 1 for from one to three minutes, the time to be estimated by experience.
2. Wash, by dipping the slide into water until the surface stain is removed.
3. Transfer to solution 2 for from three to five minutes or longer.

4. Wash, by dipping the slide into water from 1 to 3 times *only*.

5. Transfer the slide to 70 per cent alcohol, dipping hurriedly about 3 times.

6. Transfer to 80 per cent and then to 95 per cent alcohol dipping the slide into each 5 to 8 times.

7. Dehydrate in absolute alcohol for several minutes.

8. Clear in xylol and mount.

It has been found that two minutes is the shortest time that sections can be immersed in solution 2 for good results. If a two minute immersion is used, eliminate step 4 and proceed directly with step 5. If sections are left for a longer time in solution 2, immersion in water is necessary (step 4). Since the anilin blue is readily soluble in water, the depth of this stain can be controlled by the amount of washing.

OVA, Method of Examining Urine for Helminth Eggs, Barlow, C. H. Am. J. Hyg. **14**: 212, 1931.

The necessary apparatus is simple and inexpensive. It consists of straight-sided tin cans, tightly soldered and furnished with closely fitting covers; an agitating plunger; graduated cylinders on base, for measuring; pipettes for drawing off urine or a suction pump for the same purpose; centrifuge, with 50 c.c. and 15 c.c. tubes; tube rack for six 50 c.c. tubes; tube rack for twelve 15 c.c. tubes; drying cabinet or rack; slide trays for carrying slides flat; grease pencil; glass slides 38 by 75 mm. in size; and a methylene blue dropping bottle.

The urine is collected in the tin cans, which are brought to the laboratory in galvanized iron boxes holding a dozen sets of cans. Each set consists of a pair of cans, one for a stool sample and one for urine. Each pair is numbered alike for the convenience of the individual contributing the samples and also with a serial number in order to facilitate recording. A can holds 500 c.c. which is more than the average single full urination.

A common aluminum pancake turner or skimmer, which can be purchased at any five and ten cent store, is cut to fit loosely the inside of the can, and the handle is bent at right angles to the skimmer surface. This makes an agitator which thoroughly stirs the urine; but does not set up centrifugal currents but distributes the eggs evenly throughout the urine.

The urine is first measured and then poured back into the cans. It is thoroughly stirred with the agitator, held in the left hand, while it is being drawn off in a bulb pipette held in the right hand. For routine work it was found that two 50 c.c. tubes were needed in order to pick up eggs in light infestations and that a pipette holding just 50 c.c. was convenient. To prevent eggs from sticking to the sides, the pipettes must all be drawn so that there is a gradual lessening of the bore. It is essential to wash the pipettes thoroughly between each sampling by drawing up and then expelling clean water at least three times.

For known heavy or medium infestations, tubes and pipettes of 15 c.c. capacity are desirable, as the amount of centrifugate is decreased and the count is more easily made. Each tube of agitated urine is marked with the same legend as appears on the tin can from which it was taken.

The tubes are centrifugated at 800 r.p.m. for one minute and then returned to the tube racks. With a cleaned pipette the supernatant urine is carefully drawn off so as not to roil the centrifugate. This may be done speedily and safely by using an ordinary suction pump attached to the sink and carrying a pipette on a rubber tube at a fixed distance from the bottom of the tubes as they stand in the rack. This obviates too constant attendance upon the tubes, and, since the suction is steadily maintained, there is no danger of roiling the centrifugate.

The remaining urine is drawn off with a small hand pipette. Just enough urine is left in the end of the tube to spread well on the slide. A drop of aqueous solution of methylene blue is added to this.

Slides 38 × 75 mm. in size are prepared to receive the blued centrifugate by marking off on them, with a grease pencil, a parallelogram large enough to contain the centrifugate

spread evenly and not too thickly. The slide should bear the same legend as the collecting can and the tube from which the centrifugate came.

The blued centrifugate in the tubes is stirred by drawing it up into a scrupulously clean pipette and ejecting it, repeating the process a few times. Care should be taken not to get bubbles of air into the pipette. All of the centrifugate is then transferred to the greased enclosure on the slide, and the slide is set aside to dry. In the Egyptian climate the drying is rapid and no special apparatus is needed. In climates which are moist any simple drying rack or oven may be used.

STAIN: Differential Stain for Diagnosis of Neisserian Infection, Scudder, S. A. Stain Tech. 6: 99, 1931.

Films are made from exudate which has been skillfully selected. The exudate is collected by means of sterile cotton swabs, which are rolled (not rubbed or dragged) over clean new glass slides. The films are air dried, fixed by means of gentle heating and are stained in the following solutions. (Heat fixation gave slightly better results than fixation by means of alcohols.)

1. Flush the slide with buffered crystal violet solution three to five minutes.

Crystal violet	1 gm.
Distilled water	90 c.c.
Buffer solution (P_H 6.6 to 7.0)	10 c.c.

The phosphate buffer is prepared according to the method of Clark and Lubs.

2. Decant and flush with iodine solution so that the dye-iodine precipitate overflows. Time one and one-half to two minutes.

Resublimed iodine	2 gm.
Distilled water	90 c.c.
N/1 NaOH	10 c.c.

Solution of iodine in phosphate buffer was unsatisfactory.

2. Decant and decolorize by means of Merck's pure technical acetone, adding the acetone drop by drop until the drippings are colorless. Time ten seconds or less. Air dry.

4. Counterstain with the following differential methyl-green-pyronin mixture one and one-half to two minutes. Prepare counterstain a day or two before using. This counterstain can be employed in examination of formalized tissue also (see Scudder and Lisa, 1931).

Pyronin yellowish	0.1 gm.
Over ethylated methyl green	1.25 gm.
Distilled water (hot)	99 c.c.

Wash quickly with tap water. Air dry and examine by means of oil immersion lens and artificial daylight. Oil of bergamot is preferable to xylene for clearing the slides.

Gram-positive bacteria are purple black. The majority of gram-negative bacteria are faded pink; however, a small influenzal bacillus which has frequently been encountered in exudate stains deep magenta. The capsule of Friedlander's bacillus stains a transparent lavender.

The polynuclear nucleus is dark blue purple, the cytoplasm faint lavender. The lymphocyte nucleus is homogeneous very deep clear blue, the cytoplasm faint magenta violet to magenta. The plasma cell nucleus is dark navy blue with cart wheel arrangement, the cytoplasm homogeneous deep magenta to purple-magenta. The eosinophile nucleus is like that of the polynuclear and granules are fairly distinct reddish and occasionally purplish. The endothelial nucleus is pale blue, the cytoplasm pinkish lavender. The red blood cell is pale magenta to bronze. Bronzing of the red blood cell is due to impregnation of dilute iodine. The nucleus of the squamous epithelial cell is purple, the cytoplasm lavender. Purplish tinting of the epithelium is due to retention of dilute crystal violet dye. The nucleus of the columnar epithelial cell of the urinary tract is more blue than that of other epithelium and

the cytoplasm stains more deeply magenta. Retention of pure methyl green gives the blue tinting and retention of dilute pyronin gives the magenta tinting. The phagocytic endothelioid cell is more reddish and more finely granular than other cells and may be found packed with gonococci in the free exudate from cases of gonorrhea.

PREGNANCY, Serum Diagnosis of, Bebchuk, S. L. Vrach. Gaz. Leningrad 3: 189, 1931.

The author uses the Mertz test.

To 1 c.c. of blood serum (hemolytic serum might be used as well) he adds 1 c.c. of the reagent (2.8:1,000) and leaves the tubes for half an hour at room temperature. During this time the serum of the pregnant women becomes turbid. One might add after half an hour two or three drops of bromophenol as an indicator; the serum of the pregnant will turn light blue while the normal serum will remain clear. Centrifugation is not necessary. On the basis of 303 cases observed (216 women pregnant, 20 nursing, 10 healthy with delayed menses, 23 with gynecologic disorders, 19 with cancer of various pelvic organs and 15 males) he found that the reaction is positive in 90.2 per cent of all pregnant patients. The author thinks that the second part of the reaction is not of great value and with bromophenol unavailable it may be omitted. The hemolytic serum is, of course, of no use for the color part of the reaction. Mertz' reaction is of great diagnostic value in almost 90 per cent of all patients with delayed menses, although it does not explain the causes of such a delay and is undoubtedly unable to give any definite points in the differential diagnosis. The reaction is distinctly noteworthy because of its simplicity and easy application in dispensary and office practice.

Tissue, Preparation and Staining Large Bone Sections, Wagoner, G. J. Bone & Joint Surg. 13: 325, 1931.

Two methods of decalcification have been selected following extensive trial with many.

Nitric Acid: a 5 per cent aqueous solution of nitric acid will adequately decalcify large bone specimens within twenty days. The acid is changed frequently, as are the positions of the sections immersed in it.

Formic-citrate—formic-citrate solution consists of equal parts of: (1) 50 per cent aqueous solution of formic acid, (2) 20 per cent aqueous solution of sodium citrate. When this solvent is used, the solution is changed frequently and the position of the sections altered. Decalcification is complete in from ten to fifteen days. With the use of formic solution for decalcification there is less destruction of the cellular elements of the bone and less impairment of their staining qualities than result when nitric acid is used.

The stage of decalcification is tested either by needling the specimen, or better by shaving it with a sharp knife or razor. Accurate estimation of the process of decalcification may be obtained by the daily determination of the hydrogen-ion content of the solvent.

Neutralization: If decalcification has proceeded in nitric acid, the specimen is immersed in a 5 per cent aqueous solution of potassium alum for twenty-four hours. This stage is omitted if formic citrate is employed.

Washing: The decalcified bone specimen is washed with running tap water for twenty-four hours.

Dehydration: The bone is completely dehydrated by immersing for twenty-four hours in each of a number of solutions. These solutions consist of 70 per cent alcohol, 95 per cent alcohol, absolute alcohol, and three changes of ether-alcohol. The ether-alcohol consists of equal parts of ether and absolute alcohol. The absolute alcohol and ether used are kept water-free by overlaying a stratum of anhydrous copper sulphate.

Infiltration: Infiltration is with celloidin. Although the best results have been obtained with the use of Schering's celloidin, it has been too expensive for general use. Good results are routinely obtained by the use of Du Pont's parlodion.

Throughout the entire process of infiltration great care must be taken to maintain anhydrous conditions. The celloidin must only be made from clean, thin, oven-dried celloidin

shavings. All glassware, and utensils used in the preparation of the celloidin solutions must be thoroughly dried in the hot-air oven before their use. The ether-alcohol used as a solvent for the celloidin must be anhydrous.

Five solutions of celloidin are employed. These vary in viscosity from that of No. 1 which is water thin to No. 5 which is of the thickness of honey. Each celloidin solution is kept in an air-tight container.

The thoroughly dehydrated bone specimen is left in the first celloidin solution for three weeks. It remains in the second solution for two weeks and in each of the remaining three solutions for one week.

Evaporation: After the specimen has remained in the last celloidin solution for one week, it is overlaid by a volume of the thickest celloidin solution equal to approximately five times the thickness of the specimen. Evaporation is now carried out very slowly and with care to avoid bubbles. The imbedding or evaporating dish is kept in a cool place. As the consistency of the celloidin increases, it is first freed from the walls of the container with a knife. Next a large block containing the specimen is outlined, and as the consistency increases the block is gradually freed from the surrounding mass and from day to day turned on its various faces. When the various faces of the celloidin block are of equal consistency and the whole has the feel of dense rubber, further hardening is carried on by immersing the block in 65 per cent alcohol. If the above process is properly executed, the celloidin block will be found to be exceedingly hard and transparent. Any milkiness which may be present is indicative of the presence of water and will impair the final result.

BLOCKING

The hardened celloidin block is trimmed to desired shape, leaving a margin of at least five millimeters of celloidin about the tissue. It is mounted on a dry fiber block in the following manner:

1. Immerse celloidin block in 95 per cent alcohol.
2. Immerse celloidin block in absolute alcohol.
3. Immerse celloidin block in absolute alcohol and ether (equal parts) until the surface feels slimy.
4. Submerge celloidin block in thick celloidin solution and transfer quickly to the fiber block.
5. Allow to stand in air until a thick scum forms over the celloidin (ten minutes).
6. Trim away excess from margin of fiber block and immerse in 70 per cent alcohol for at least twelve hours before cutting.

The cut sections are stained with Weigert's iron hematoxylin and counterstained with eosin according to the following technic.

WEIGERT'S IRON HEMATOXYLIN

Solution A:	
Hematoxylin	1 gm.
Alcohol 95 per cent	100 c.c.
Solution B:	
Distilled water	95 c.c.
Solution iron chloride	4 c.c.
Hydrochloric acid	1 c.c.

Mix equal parts of Solutions A and B each time it is to be used.

From the 70 per cent alcohol in which the sections were placed when cut, the staining process continues by immersing in:

1. Water, to remove alcohol.
2. Hematoxylin, five minutes.
3. Water, to remove excess hematoxylin.
4. Acid alcohol, until celloidin clears.

5. Water, to remove acid alcohol.
6. Ammonia water, five to thirty minutes.
7. Water, dip.
8. Eosin, dip.
9. Water, to remove excess eosin.
10. Alcohol 70 per cent, one minute.
11. Alcohol 95 per cent, until celloidin clears.
12. Creosote.

Mount from the creosote in Canada balsam on clean slides and cover. The mounted section is laid on a smooth surface and a heavy weight placed on the cover slip. This weight is allowed to remain in place for two to three days until the balsam is well set. Such a procedure aids in pressing out any wrinkles in the section and in freeing it from inclusions of air bubbles.

Lantern Slides: Sections of 25 microns' thickness may be stained and mounted in balsam between the two glass plates of the ordinary lantern slide. The slide is then placed in the oven used in the routine paraffin technic and allowed to remain for from three to four days. At the end of this time the balsam will be quite firm and the slide is bound and the mat applied. There results from this procedure an excellent colored lantern slide of the desired section.

Colloidal Gold: Preparation of, Patterson, J. Brit. J. Exper. Med. **12**: 143, 1931.

Reagents:

1. Distilled water.
2. Potassium oxalate (of analytical purity) 1 per cent solution.
3. Gold-sodium chloride (double salt), $AuCl_3$, NaCl, $2H_2O$.
4. N/50 sodium hydroxide.
5. N/50 hydrochloric acid.

Notes on reagents: The water is prepared by distilling ordinary tap or distilled water through an all-glass (hard or resistance glass) apparatus rejecting the first 5 per cent, leaving a similar amount as residue and collecting the mid-fraction for use. Although this glass-distilled water has been used in the standard preparation, a gold sol nearly equal in quality results from the substitution of the ordinary laboratory distilled water furnished by the usual type of large copper still.

The oxalate is dissolved in ordinary distilled water, and is used as long as it is perfectly clear. In time a haze or slight precipitate develops when a fresh solution is made up. For the gold chloride the sodium double salt has been used as the standard reagent, but the acid salt functions equally well, the necessary adjustment corresponding to the change of gold being made apparent in the preliminary test.

The alkali is the ordinary N/50 standard solution prepared from a stock CO_2 free sodium hydroxide, and preserved in a hard-glass bottle.

THE METHOD

Preliminary Test: To 50 c.c. of the distilled water 0.5 c.c. of 1 per cent potassium oxalate and 0.5 c.c. of gold chloride solution are added. A row of test tubes is set up and 5 c.c. of the mixture placed in each. The first of the series being left as made up, 1 drop of N/50 alkali is added to the second, 2 drops to the third, and so on to the sixth, a standard dropping pipette being employed (one giving 25 drops to the c.c.). The whole series of tubes is then immediately immersed in about 70 c.c. of water at air temperature contained in a 250 c.c. hard-glass beaker. The temperature of the water-bath is then rapidly raised, heating being by means of a strong Bunsen burner under a thin plain iron wire gauze (i.e., without an asbestos center). When the bath has reached boiling point and remained there for about a minute, the several tubes are withdrawn and placed in order in a rack. Of these tubes only one is representative of the right conditions for the preparation of the

stock gold sol. It is the lowest in the series to give a bright red clear solution which, when carefully examined in a good light, exhibits just the faintest sheen. Those containing less alkali eliminate themselves as exhibiting a very marked sheen, while those containing more alkali than the selected tube are too clear and purple, the total color development being also less intense. In general, when the sodium gold chloride double salt has been used tube 4 is representative of the correct conditions. It is equivalent to the use of 1 c.c. of gold chloride (1 per cent solution), 1 c.c. of 1 per cent potassium oxalate and 2.4 c.c. of N/50 sodium hydroxide with each 100 c.c. of distilled water.

The way is now open to the preparation of the gold solution itself in bulk.

Actual Preparation of the Stock Gold Solution: To 200 c.c. of distilled water contained in a hard-glass beaker (just previously treated with aqua regia, and washed with successive quantities of tap water and distilled water) 2 c.c. of 1 per cent potassium oxalate, 2 c.c. of 1 per cent gold chloride and 4.8 c.c. (or such amount as has been ascertained in the preliminary test) of N/50 alkali are added. The whole is raised rapidly to the boiling point. The initial change in the formation of the hydrosol is indicated by the appearance of a light blue color, which soon darkens, and then as the temperature is further raised, the solution, still remaining clear, becomes more and more purple, then red, until just before the boiling point is reached a rapid final change to a light cherry red sets in, the sol then remaining perfectly stable in color after the boiling point is attained. Once this final stage is reached the heat is withdrawn, the beaker covered and allowed to cool. When sufficiently cool it is transferred to a glass-stoppered stock bottle.

Titration of Stock Gold Solution: A few drops of blood are taken directly from the finger into about 10 c.c. of normal saline, the red corpuscles spun down, and after the supernatant liquid is removed, are washed twice with 10 c.c. of saline. A 1 in 100 dilution of the corpuscles is made in water, and is then used upon the gold solution exactly as a specimen of cerebrospinal fluid is used in the Lange test (i.e., with 2.5 c.c. of gold sol to each tube). The test is made against four different specimens of gold sol acidified as follows:

Specimen	*Amount of stock sol.*	*Volume of acid added*
1	20 c.c.	0.35 c.c. N/50 HCl
2	20 c.c.	0.40 c.c. N/50 HCl
3	20 c.c.	0.45 c.c. N/50 HCl
4	20 c.c.	0.50 c.c. N/50 HCl

Readings are made after twenty-four hours.

With the smallest amount of acid it is usually found that the first five tubes of the Lange series are not affected, whereas with the greatest amount of acid all five will show complete precipitation. The correct addition of acid is the smallest quantity that will just enable the gold to be partially precipitated by the oxyhemoglobin, the tubes 1 to 5, to which attention is confined, showing a pale pink color. (The later tubes of the series, particularly 6 and 7, show precipitation at this stage, and even with slightly lower additions of acid, but this precipitation is of a different type to that just mentioned, the obvious difference is that it leads to a bluish-purple color instead of a pink, and need not be taken into consideration for the purpose of defining the end-point of the titration.) In effect this is really a titration to P_H 6.7-6.8, the isoelectric point of oxyhemoglobin, which P_H refers, however, not to the gold sol itself, but to the mixture when all the reacting substances are together.

With very little experience it is possible to ascertain the correct acid addition by noting the appearance of the tubes after ten minutes, without waiting for the final reading at twenty-four hours, and also to cut down the number of specimens of acidified gold to be tested to two. It is essential to use a freshly prepared solution of oxyhemoglobin for the titration.

Immediately before use the stock gold sol is acidified in accordance with the above titration, and is then completely standardized, fulfilling all the requirements of the Lange reaction.

PREGNANCY, Rapid Method for Diagnosis of, Eberson, F. and Silverberg, M. H. J. A. M. A. **96:** 2176, 1931.

From 6 to 8 ounces (180 to 235 c.c.) of morning urine was obtained and 180 c.c. used in the test, as follows:

Two and one-half volumes of 95 per cent alcohol were added to the urine, and the mixture was kept in an ice-chest at a temperature of from 2 to 4° C. for several hours or overnight to allow the precipitate to settle out. In order to hasten the preliminary procedures, frequently the mixture was centrifugated and the sediment treated as follows: The precipitate, after centrifugation, was washed several times with from 10 to 15 c.c. of ether and dried in the incubator at from 37.5 to 38° C. or by means of a stream of compressed air. A more satisfactory procedure was adopted by adding the ether to a suspension of the sediment in 6 c.c. of physiologic solution of sodium chloride shaking the mixture thoroughly, centrifugating it to remove the supernatant ether, and repeating the extraction two or three times. The supernatant saline solution after centrifugating and containing the specific hormone, now freed from the estrous or ovarian hormone, was used for injection into rats.

Female immature rats from eighteen to twenty-one days old, taken from one litter, were used in the test. One cubic centimeter of the extract was injected into each of two rats on three successive days. On the third or fourth day, the animals, including an untreated normal control, were killed. The reproductive tract was examined in situ, and special attention was given to the appearance of these organs. Serial sections were prepared from the ovaries, tubes, and uterus for microscopic confirmation of gross conditions.

The technic has been improved recently to the extent of injecting 1 c.c. of the test material twice a day for one or two days, thus shortening the time required for diagnosis to from thirty-six to forty-eight hours, instead of from ninety-six to one hundred hours. By this method the precipitate obtained from the urine is suspended in 3 c.c. of salt solution and the more highly concentrated material used for injection.

Economy in the use of alcohol for this test could be practiced by distilling the residual urine-alcohol mixture and using the alcohol repeatedly. In our experience there have been no false results in numerous instances in which such reclaimed alcohol served as the reagent.

With the technic outlined there has been no mortality among the rats in the course of injections prior to the death of the animals.

INTERPRETATION OF TEST

The gross appearance of the ovaries, tubes and uterus was carefully noted. The organs, in positive tests, were uniformly enlarged, and the tubes, in particular, were distended and translucent in appearance, owing to the contained fluid. The ovaries were scrutinized for hemorrhagic protruding follicles and "blood points." In the positive instances the ovaries were unmistakably enlarged, hemorrhagic and congested. The ovarian and tubal blood vessels were engorged and stood out in bold relief as compared with normal controls. The uterus was invariably hypertrophied and turbid. In mildly positive cases seen from time to time in very early pregnancies, the gross picture was convincing despite the less pronounced changes.

Microscopically, the diagnostic criteria were the enlarged hemorrhagic follicles containing corpora lutea. The degree of luteinization varied from slight invasion at the periphery to complete transformation filling the entire structure. As will be noted subsequently, the extent of these specific changes and their progressive or retrogressive aspects were modified by the age of the fetus and certain other clinical factors. In the absence of grossly positive signs, the microscopic picture always determined the diagnosis. The presence of at least one corpus luteum was required for a positive diagnosis.

TISSUE, Rapid and Permanent Stain for Myelin Sheaths, Courville, C. and Krajian, A. Arch. Path. **11:** 920, 1931.

Hardening: For rapid diagnosis on fresh tissue, boil the blocks for one minute in 10 per cent formaldehyde and then leave in the same solution in a paraffin oven for five min-

utes before cutting frozen sections. When the section is not demanded in a short time, fix the tissues in 10 per cent formaldehyde for twenty-four hours or longer.

Sections: Cut frozen sections 10 microns thick. Receive sections in a large dish containing tap water.

Mounting: Draw sections on to slides and let the excess water drain off for about a minute and dehydrate with absolute alcohol, pouring on a few drops two or three times, each time allowing it to evaporate. Blot and dip the slide in a thin celloidin fixing the section to the slide. Dip the slide in tap water for a few seconds.

Mordanting: Cover the section with 15 per cent hot aqueous solution of ferric chloride (Ferric chloride solution should be made up fresh each time. Dissolve 15 gm. of ferric chloride in 100 c.c. of hot tap water and raise the temperature to 60° C. just before use) for five minutes. Drain the solution from the slide. Do not wash.

Staining: Cover section for five minutes with equal parts of hematoxylin solution (Hematoxylin solution is prepared by dissolving 10 gm. of hematoxylin crystals in 90 c.c. of absolute alcohol and ripening in an incubator at 37° C. for two or three weeks. This solution is stable and will keep for months) and distilled water, heated to 60° C. This stains the section extremely black.

Washing: Wash thoroughly in tap water.

Destaining: Remove excess stain in a 1 per cent aqueous solution of ferric chloride, dipping the slide in and out of the solution until the gray matter begins to appear in contrast to the dark medullary substance. This takes place in from ten to twenty-five seconds.

Washing: Wash the slide quickly in tap water.

Differentiation: Cover the section with a 0.25 per cent aqueous solution of potassium permanganate and shake with the fingers to secure even differentiation which takes place in about five seconds. This step should be controlled under the microscope.

Washing: Thoroughly wash the section in tap water.

Dehydration: Remove water from the section after draining off the excess by pouring on a few drops of absolute alcohol and draining off, repeating three or four times. Blot between filter paper.

Clearing: Plunge section in a container of equal parts of aniline oil and xylene for about three minutes and then in xylene for another three minutes.

Mounting: Mount in Canada balsam or gum damar.

BLOOD CHLORIDES: Determination of Using Palladious as Indicator, Lewis, R. C., and Binkley, N. L. Am. J. Clin. Path. **1**: 231, 1931.

SOLUTIONS REQUIRED

1. Sulphosalicylic acid. A 2 per cent solution.
2. Standard silver nitrate solution (1 c.c.-1.28 mg. NaCl).

Silver nitrate, c.p.	3.72	grams
Nitric acid, conc.	250	c.c.
Water to make	1000	c.c.

3. Standard potassium iodide solution (2 c.c. to 1 c.c. Standard $AgNO_3$) Transfer approximately 1.9 gm. of potassium iodide to a liter volumetric flask, dissolve in water, and dilute to volume. Measure 1 c.c. of the standard silver nitrate solution, 9 c.c. of water, and 0.2 c.c. of palladious nitrate indicator into a small Erlenmeyer flask and titrate from a micro-burette with the potassium iodide solution to the first permanent brown color. After checking the titration, adjust the solution by dilution so that exactly 2.03 c.c. instead of 2 c.c. of the potassium iodide solution will be required to titrate 1 c.c. of the standard silver nitrate solution to the brown end-point with palladious nitrate indicator. The extra 0.03 c.c. of potassium iodide solution is necessary to provide for the blank required to produce the end-point with the indicator. When this standard potassium iodide solution is used in the titration of excess silver nitrate in blood chloride determinations, the same blank of 0.03 c.c. is subtracted from the titration value obtained.

4. Palladious nitrate indicator solution.

Palladious nitrate	0.13	gram
Nitric acid, conc.	16	c.c.
Water to make	100	c.c.

This solution keeps indefinitely.

TECHNIC OF METHOD

To 2 c.c. of plasma or whole blood in a 25 c.c. volumetric flask add about 6 c.c. of water, and then 15 c.c. of 2 per cent sulphosalicylic acid. Dilute to volume, shake, allow to stand five to ten minutes, and filter. To 10 c.c. of the water-clear filtrate in a 25 c.c. volumetric flask, add 5 c.c. of standard silver nitrate solution, and dilute to volume. Add a small pinch of kaolin to aid the coagulation of the silver chloride formed, shake thoroughly, allow to stand five to ten minutes, and filter. If the first few drops of the filtrate are cloudy, pour back through the filter to obtain a clear solution. To 10 c.c. of the filtrate in a small Erlenmeyer flask, add 0.2 c.c. of palladious nitrate indicator and, using a micro-burette, titrate with standard potassium iodide to the first brown color. The end-point is very distinct and permanent.

CALCULATION OF RESULTS

The amount of sodium chloride present may be calculated from the following formula:

$$\text{Milligram of NaCl per 100 c.c. of blood (or plasma)} = 2 - \frac{\text{KI used} - \text{titration blank}}{2}$$

$$\times \text{ mg. of NaCl per c.c. of } AgNO_3 \times \frac{100}{\text{Blood (or plasma) equivalent of filtrate used}}$$

$$= \frac{4 - (\text{KI used} - 0.03)}{2} \times 1.28 \times \frac{100}{0.32} = 4 - (\text{KI used} - 0.03) \times 200.$$

Thus, to find the number of milligrams of sodium chloride in 100 c.c. of plasma (or whole blood), subtract 0.03 (the titration blank) from the number of cubic centimeters of KI used, then subtract this figure from 4, and multiply the difference obtained by 200.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Candiru**

HERE is a story, a medical story, that will make your blood run cold just as it did when you were a child and your negro mammy told you all about raw beef and bloody bones. When I was a child reading geography I thought how terrible it would be to be swimming in the Amazon River and have a boa constrictor drop down upon me from the trees. It seems the natives have a terror of swimming in the river but not from boa constrictors. Instead it is a very harmless looking little fish about one or two inches long called the Candiru. Unfortunately this animal is urinophilous and is strongly attracted to urine when voided in the river. Indeed, it is so strongly attracted that it will penetrate the urinary meatus. Then, of course trouble begins.

These stories of the Candiru have been more or less legendary and there have been few investigators who have actually seen the event.

Gudger who is in the department of Ichthyology in the American Museum of Natural History has made a very complete and very interesting study of the investigations that have been made in an attempt to substantiate or refute the story of the Candiru. His conclusions appear to be justified by the evidence that he presents.

An Index to the Chemical Action of Microorganisms†

THIS is a reference index which should be of great service to the bacteriologist and the chemist. It consists of three cross index tables. The first index lists the microorganisms that have been studied. Under each microorganism is listed those substances which have been used as substrates and in the next column there appear the chemical substances that are produced as a result of the action of the microorganism on the substrate. The final column gives the reference to the authors who did the experimental work. Thus, aspergillus niger grown on citric acid produces glycollic acid according to the report of Challenger, Subramaniam and Walker, in 1927. The same organism grown on oxalic acid also produces glycollic acid according to the same authors. Grown on sucrose it produces citric acid according to Currie, 1917.

The second table tells the reader what may be produced on a certain substrate with specific organisms. Thus, acetic acid may be used for the preparation of formaldehyde and

*The Candiru. The Only Vertebrate Parasite of Man. By Eugene Willis Gudger, Ph.D. Bibliographer and Associate in Ichthyology, American Museum of Natural History, New York City. With a Foreword by Aldred Scott Warthin, Ph.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor. With 18 Illustrations. Cloth. Pages 120. Paul B. Hoeber, Inc. New York. 1930.

†An Index to the Chemical Action of Microorganisms on the Non-Nitrogenous Organic Compounds. By Ellis I. Fulmer, Ph.D., Professor of Biophysical Chemistry, Iowa State College and C. H. Werkman, Ph.D., Associate Professor of Bacteriology, Iowa State College, Assistant Chief in Bacteriology Iowa Agricultural Experiment Station. Assisted by Anella Wieben and Calvin R. Breden, Instructors in Chemistry, Iowa State College. Cloth. Pages 198. Charles C. Thomas, Springfield, Illinois, 1930.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

formic acid by the growth of bacillus pyocyaneus according to Submiewski, 1913. Acetic acid is a substrate for the production of glycollic acid as a result of the growth of aspergillus niger according to Challenger, Subramaniam and Walker, 1927. Acetone as a substrate will produce acetic acid and formic acid following the growth of bacillus pyocyaneus according to Submiewski, 1923.

The third table again cross indexes, carrying the product as the major index. Dextrin may be produced from starch by B. amylobacter, according to Villiers, 1891. Glycerol is produced from dextrose by the action of yeast (Oppenheimer, 1913). The final table consists of the references employed in the first three.

This volume should have a very definite value to research students interested in this field.

---

*The Use of the Microscope**

THIS is a little out of the usual line. It is in no sense an elementary book but is of particular interest to those who have used the microscope for a long time and who feel that they are well acquainted with all of its idiosynerasies. The average microscopist probably only obtains about 50 per cent of efficiency in magnification and detail from the use of his microscope. This volume, contributed by the cytologist of the Carnegie Institution, describes in detail the common errors in the use of the condenser, the illumination, the filters and screen, cover glasses, objective, mirrors, eye pieces, etc., and details the necessary steps to obtain maximum efficiency with the microscope.

For routine work one may possibly be satisfied with 50 per cent efficiency but when we are dealing with experimental investigation this will not do. For example, a man who is making special studies of chromosomes and even of chromomeres must provide maximum detail in order to obtain results that are at all satisfactory.

In essence the volume is a treatise on the physics of light as applied to microscopy, but we will venture to say that most of the discussion deals with minutiae which are unknown to the average microscopist. For those who are interested in improving their microscopic acumen, the work may be highly recommended.

X-Ray Technology†

THIS is a very advanced volume on the physics of the x-ray and roentgenology, of interest to the roentgenologist who desires to obtain maximum efficiency in his work. The leading roentgenologists find that they must have expert physicists in association, particularly when dealing with high voltage machines used especially in therapy. The writer has prepared this volume for the roentgenologist to serve in lieu of the expert services of a physicist, where the latter is not available.

The Creed of a Biologist‡

THE trouble with the attitude of present-day scientific men toward orthodox religion is that their attitude is negative in character. They find that they cannot believe in revealed religion, but with a few exceptions a thoroughly satisfying positive creed has not been built up in its place. Dr. Warthin's most inspiring essay creates a positive religion based

---

*The Use of the Microscope. A Handbook for Routine and Research Work. By John Belling. Cytologist, Carnegie Institution of Washington. First Edition. Cloth. Pages 315. McGraw-Hill Book Company, Inc. New York, 1930.

†X-Ray Technology. The Production, Measurement and Applications of X-Rays. By H. M. Terrill, Ph.D., Associate in Physics, Institute of Cancer Research and C. T. Ulrey, Ph.D., Research Physicist, Westinghouse Lamp Company. Cloth. Pages 256. New York. D. Van Nostrand Company, Inc., 1930.

‡The Creed of a Biologist. A Biologic Philosophy of Life. By Aldred Scott Warthin, Ph.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor. Cloth. Pages 60. Paul B. Hoeber, Inc., New York, 1929.

upon the scientific knowledge of today. It is, of course, that which most intellectual thinkers have accepted, namely the submersion of any idea or hope for personal immortality in the recognition of racial immortality. Dr. Warthin has built up a series of rules for conduct based on his religious interpretations. Race betterment through eugenic means naturally becomes a prominent factor.

The essay is most stimulating.

---

*Bedside Interpretation of Laboratory Findings**

THIS is the type of thing the general man wants. Having received such and such a report from the laboratory, how to interpret it? Dr. Wohl has done a good job in a relatively small space. His interpretations are brief, to the point and indicate an acquaintance with the current literature right up to the time of publication.

No two people would write such a book alike. Some would include more, others less. But the volume under review appears to fulfill the promise of the title very satisfactorily.

Sometimes the writer includes more than the title requires. Thus, treatment is often included. This is unobjectionable, but sometimes when one gets into the phase of treatment one finds it necessary to devote much more space than is available, in order to give it its due. For example, the author takes up desensitization treatment in allergy. This being a sideline from the main purpose of the book it is very brief and could not be followed as described without occasional risk of reaction.

A general practitioner who has received a formal laboratory report and who has some hesitancy in asking what he feels may be too simple questions will be able to dig out the answer for himself without trouble with the aid of this volume.

Practical Physiological Chemistry†

THE fourth edition of Zinsser's standard work requires no introduction. Developments ber. The tenth edition marks the twenty-fifth anniversary of the appearance of Hawk's Physiological Chemistry. This latest edition has been brought up to date with revision and resetting of much of the book and rewriting of the sections on Examination of Blood and Urine, Physical-Chemical Properties of Solutions, Enzymes and Their Action, Endocrin Organs, and Proteins.

The general construction of the volume remains, as formerly, that of a practical laboratory and reference manual designed primarily as a textbook for undergraduates in physiological chemistry and serving at the same time as a reference volume for graduates.

Resistance to Infectious Diseases ‡

THE fourth edition of Zinsser's standard work requires no introduction. Developments have been so rapid in the field of immunology and viewpoints have changed so rapidly that a book rapidly becomes out of date. As the autnor remarks in his preface, "In the subject of immunology seven or eight years suffice to render a young book decrepit. One

---

*Bedside Interpretation of Laboratory Findings. By Michael G. Wohl, M.D., Associate Professor of Experimental Medicine, Temple University Medical School. Introduction by Joseph McFarland, M.D., Sc.D. Professor of Pathology, University of Pennsylvania. Illustrated. Cloth. Pages 321. St. Louis. The C. V. Mosby Co., 1931.

†Practical Physiological Chemistry. A Book Designed for Use in Courses in Practical Physiological Chemistry in Schools of Medicine and of Science. By Philip B. Hawk, M.S., Ph.D., President of the Food Research Laboratories, Inc., New York City, and Olaf Bergeim, M.S., Ph.D., Associate Professor of Physiological Chemistry in the University of Illinois, College of Medicine, Chicago, Tenth (25 anniversary) Edition. Rewritten and Reset. With 2 full page plates of absorption spectra in colors—6 additional full page color plates and 280 figures of which twelve are in colors. Cloth. Pages 929. Philadelphia, P. Blakiston's Son and Co., Inc., 1931.

‡Resistance to Infectious Diseases. By Hans Zinsser, M.D., Profesor of Bacteriology and Immunity, Medical School, Harvard University. Fourth Edition, Completely Revised and Reset. Cloth. Pages 651. New York. The Macmillan Company, 1931.

who has rashly undertaken the task of presenting this subject in book form is thereafter condemned to the alternatives of either the periodical labor of revision or a sense of guilty responsibility for many views and conceptions which he no longer supports in their original forms, unless he has become as relatively old as the book itself. Books on immunology should either be revised every ten years or destroyed except for a few museum copies.''

Investigation in the field, in the last few years has been made with a far greater degree of experimental precision than in the earlier days of immunology and the more recent developments have been based upon more accurately controlled observations than those previously founded upon the trial and error methods ot pure biology. The author states that this has necessitated comprehensive revision, and, while the fundamentals of the book remain unchanged, much of it has been rewritten, bringing it entirely up to date.

With a volume such as this, which deals with a constantly growing and changing subject, the appearance of a new edition at once means that preceding editions are thereafter chiefly of historical interest.

---

*The Principles of Bacteriology and Immunity**

THIS is a comprehensive book in two volumes, the standard British reference volume on this subject. The discussion of laboratory bacteriology and immunology and the review of the general subject of anaphylaxis, infection and resistance are both comprehensive and authoritative. This work has an advantage over most of the other presentations on this subject in that it also contains paragraphs on practical application.

It should be of distinct value to all interested in the general fields under consideration.

A Text-Book of Pathology†

IT IS refreshing to see a medical book that has had so many editions that the authors have ceased including prefaces to previous editions and have let it go at a single new preface to the fifteenth edition. In other words by now the work speaks for itself. That Delafield and Prudden will continue paramount among textbooks of pathology has been assured by the fact that as eminent a pathologist as Francis Carter Wood has charge of the revision.

It is a matter of some interest that the additions to our knowledge of pathology that have been made since the last edition appeared have been preeminently in the realm of functional pathology. These include further studies on the thyroid hormone, the correlation of parathyroid disease with osteitis fibrosa cystica, the work of Aschheim and Zondek on the pituitary secretion, the recent work on ovarian hormone, knowledge of the effect of ultraviolet irradiation on ergosterol, the preparation of a potent extract of the adrenal cortex.

But while these are the outstanding recent advances, it is equally true that a textbook of pathology more than a few years old is out of date even as regards morphologic pathology. Recent advances in our knowledge of cancer and the blood dyscrasias have contributed especially to this.

---

*The Principles of Bacteriology and Immunity. By W. W. C. Topley, M.A., M.D., M.Sc., F.R.C.P., Professor of Bacteriology and Immunology, University of London, Director of the Division of Bacteriology and Immunology, London School of Hygiene and Tropical Medicine. And G. S. Wilson, M.D., M.R.C.P., D.P.H., Reader in Bacteriology and Immunology in the University of London, London School of Hygiene and Tropical Medicine. In Two Volumes. Cloth. Pages 1,300. New York, William Wood and Company, 1929.

†A Text-Book of Pathology. By Francis Delafield, M.D., LL.D., Some time Professor of the Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York and T. Mitchell Prudden, M.D., LL.D., Some time Professor of Pathology, College of Physicians and Surgeons, Columbia University, New York. Fifteenth Edition. Revised by Francis Carter Wood, M.D., Director of the Pathological Department, St. Luke's Hospital, New York. With 20 Full-page Plates and 830 illustrations in the text in black and colors. Cloth. Pages 1,339. New York. William Wood and Company, 1931.

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., OCTOBER, 1931 NO. 1

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - - - - - LOS ANGELES
J. J. R. MACLEOD, M.B. - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - CLEVELAND, OHIO
JOHN A. KOLMER, M.D. - - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - NEW ORLEANS
T. B. MAGATH, M.D. - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - - BALTIMORE
M. H. SOULE, SC.D. - - - - - - ANN ARBOR, MICH.

Contents of this Journal Copyright, 1931, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIAL

The Specific Therapy of Pneumonia

DESPITE extensive studies in recent years pneumonia still deserves Osler's famous description of this disease as "Captain of the Men of Death."

Any studies, therefore, which shed light upon the results of attempts at specific therapy are of great interest and well repay perusal.

The report of Cecil and Plummer[1] is of importance covering as it does a careful survey of 1,161 cases of pneumococcus type I pneumonia.

While the report is best studied in the original, its high lights may be summarized with profit.

Despite the fact that the pneumococcus type I is but rarely found in healthy throats this organism is responsible for by far the greatest number of cases of lobar pneumonia (30.9 per cent of 3,662 cases). These figures refer to adult cases as type I pneumonia appears to be rare in infants and constitutes only 10 per cent of the cases occurring between the ages of three and twelve years.

[1]Cecil, R. L., and Plummer, N.: Pneumococcus Type I Pneumonia, J. A. M. A. 95: 1547, 1930.

On the other hand, as has frequently been commented upon, the disease is essentially a disease of the young, 61.8 per cent of the cases in the series occurring between ten and forty years of age.

Type I pneumonia is the prototype of lobar pneumonia in that the clinical history and course conform closely to the "typical" description.

The commonest complications in children were otitis media and empyema although various complications may be encountered in a small percentage of cases.

In the series reported it was noted that there was a steadily rising death rate from 1921 to 1929, namely, from 20 to 42.8 per cent. The reason for this is not apparent.

While many factors influence the death rate in pneumonia, among them type of patient, the incidence of alcoholism, the age and so on, Cecil and Plummer note a direct relation between the death rate and pneumococcus bacteremia, the death rate with bacteremia being 66.7 per cent and but 22.5 per cent in the absence of bacteremia.

Of particular interest is that portion of the report dealing with specific therapy.

It is, of course, well recognized that while the experimental and clinical evidence in favor of the use of type I serum is quite convincing the statistical evidence is not equally convincing. The investigations of Cecil and Plummer begun in 1920 are, therefore, of great interest especially as they utilized not only type I serum but also the antibody solution of Huntoon and the concentrated serum of Felton.

Huntoon's antibody solution is polyvalent for types I, II, and III, highly effective against type I pneumococci, less so against type II, and with but little value against virulent type III cultures.

The authors feel that while the freedom of Huntoon's solution from horse protein eliminated anaphylactic reactions and serum sickness it was no more concentrated than the original type I serum and also that while the clinical and statistical results following its use were very good, the severe thermal reactions produced were a distinct disadvantage. To this, also, may be added the fact that it is quite expensive.

Felton's concentrated serum was tested in 239 cases with excellent results and shown to be often ten times more potent than unconcentrated preparations so that this product deserves a more extensive use.

Finally, in the concluding words of the authors:

"Type I serum is no longer in the experimental stage. When administered early and in sufficient dosage, the clinical results are striking. The present study demonstrates that concentrated serum possesses all the therapeutic value of the unconcentrated preparation. Furthermore, concentrated serum has a much higher potency, and a lower content of chill-producing substances and horse serum proteins which make it more easily administered, and less frequently followed by chills, serum sickness, and serum reactions."

—*R. A. K.*